Abstracts of papers presented
at the LXXV Cold Spring Harbor Symposium
on Quantitative Biology

NUCLEAR ORGANIZATION & FUNCTION

June 2–June 7, 2010

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Abstracts of papers presented
at the LXXV Cold Spring Harbor Symposium
on Quantitative Biology

NUCLEAR ORGANIZATION & FUNCTION

June 2–June 7, 2010

Arranged by

Terri Grodzicker, *Cold Spring Harbor Laboratory*
David Spector, *Cold Spring Harbor Laboratory*
David Stewart, *Cold Spring Harbor Laboratory*
Bruce Stillman, *Cold Spring Harbor Laboratory*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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SYMPOSIUM LXXV
NUCLEAR ORGANIZATION & FUNCTION
Wednesday, June 2 – Monday, June 7, 2010

Wednesday	7:30 pm	**1** Introduction
Thursday	9:00 am	**2** Chromatin Modifications and Gene Expression
Thursday	2:00 pm	**3** Poster Session I
Thursday	4:30 pm	Wine and Cheese Party*
Thursday	7:30 pm	**4** DNA Replication & Genome Integrity
Friday	9:00 am	**5** Reprogramming & Differentiation
Friday	2:00 pm	**6** Poster Session II
Friday	7:00 pm	HARRIS LECTURE **7** RNA Biology
Saturday	9:00 am	**8** Chromosome Structure and Mitosis
Saturday	2:00 pm	**9** Heterochromatin Formation and Gene Silencing
Saturday	5:30 pm	DORCAS CUMMINGS LECTURE
Sunday	9:00 am	**10** Transcription Meets RNA Processing
Sunday	2:00 pm	**11** Poster Session III
Sunday	4:00 pm	Beach Picnic
Sunday	7:00 pm	**12** Nuclear Periphery
Monday	9:00 am	**13** Chromatin Structure and Organization
Monday	2:00 pm	**14** Genome Regulation
Monday	5:00 pm	Summary
Monday	6:00 pm	Banquet

Poster sessions are located in *Bush Lecture Hall*
* *Airslie Lawn*, weather permitting

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30am-9:00am
Lunch 11:30am-1:30pm
Dinner 5:30pm-7:00pm
Bar times are from 5:00pm until late

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PROGRAM

WEDNESDAY, June 2—7:30 PM

Welcoming Remarks

Bruce Stillman
Cold Spring Harbor Laboratory

SESSION 1 INTRODUCTION

Chairperson: **B. Stillman,** Cold Spring Harbor Laboratory, New York

Nuclear reprogramming by eggs and oocytes
J.B. Gurdon.
Presenter affiliation: University of Cambridge, Cambridge, United Kingdom. 1

Copying and reprogramming of heterochromatin with RNAi
Rob Martienssen, Keith Slotkin, Milos Tanurdzic, Matt Vaughn, Anna Kloc, Mikel Zaratiegui, Danielle Irvine, Jean-Philippe Vielle Calzada, Paul Auer, R.W. Doerge.
Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 2

Targeting X chromosomes for repression
Barbara J. Meyer.
Presenter affiliation: HHMI and University of Calfiornia Berkeley, Berkeley, California. 3

The nuclear envelope and chromatin organization
Gunter Blobel.
Presenter affiliation: Rockefeller University, New York, New York. 4

THURSDAY, June 3—9:00 AM

SESSION 2 CHROMATIN MODIFICATIONS AND GENE EXPRESSION

Chairperson: **W. Bickmore,** MRC Human Genetics Unit, Edinburgh, United Kingdom

New views of local and genome-wide transcription activation in vivo
John T. Lis, Martin Buckley, Leighton J. Core, Nickolas Fuda, Michael Guertin, Irene M. Min, Hojoong Kwak, Steven Petesch, Joshua J. Waterfall, Katie Zobeck.
Presenter affiliation: Cornell University, Ithaca, New York. 5

Steps towards understanding nuclear structure and the phenomena of epigenetics at a molecular level
Danny Reinberg.
Presenter affiliation: Howard Hughes Medical Institute, New York University School of Medicine, New York, New York. 6

Histone covalent modifications in epigenetic regulation
Shelley Berger.
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania. 7

Beyond the double helix—Varying the "histone code"
C. David Allis.
Presenter affiliation: The Rockefeller University, New York, New York. 8

Histone variant dynamics and epigenetics
Steven Henikoff, Yamini Dalal, Takehito Furuyama.
Presenter affiliation: Fred Hutchinson Cancer Research Center, Seattle, Washington. 9

Nucleosome occupancy and transcriptional regulation
Robert E. Kingston, Caroline Woo, Jonathan Dennis, Shobhit Gupta, Bill Noble, Peter Kharchenko, Peter Park.
Presenter affiliation: Massachusetts General Hospital, Boston, Massachusetts; Harvard Medical School, Boston, Massachusetts. 10

Chromatin boundaries, insulators and long range interactions in the nucleus
Gary Felsenfeld, Rodolfo Ghirlando, Keith Giles, Humaira Gowher, Chunyuan Jin, Gaelle Lefevre, Tiaojiang Xiao, Zhixiong Xu, Hongjie Yao.
Presenter affiliation: National Institutes of Health, Bethesda, Maryland. 11

THURSDAY, June 3—2:00 PM

SESSION 3 POSTER SESSION I

Flexible and precise high-resolution fluorescence imaging of chromosomal regions using complex oligonucleotide libraries
Robert A. Ach, N. Alice Yamada, Peter Tsang, Alicia Scheffer-Wong, Laurakay Bruhn.
Presenter affiliation: Agilent Technologies, Santa Clara, California. 12

MALAT-1 noncoding RNA regulates cell mobility by modulating gene expressions
Keiko Tano, Tomoko Okada, Randeep Rakwal, Kenich Ijiri, Tetsuro Hirose, Nobuyoshi Akimitsu.
Presenter affiliation: University of Tokyo, Tokyo, Japan. 13

Transcriptomic, nucleocytoplasmic, and interactodynamic analyses of the nuclear ribonucleases Rrp6 and Dis3
Erik D. Andrulis, Megan C. Mamolen, Daniel L. Kiss.
Presenter affiliation: Case Western Reserve University School of Medicine, Cleveland, Ohio. 14

Spatial organization of the P2 odorant receptor alleles within the nucleus of olfactory neurons
Lúcia M. Armelin-Correa, Débora Y. Brandt, Luciana Gutiyama, Bettina Malnic.
Presenter affiliation: Chemistry Institute–University of São Paulo, São Paulo, Brazil. 15

BEAF 32 -CP 190—A new class of insulators role in chromatin organization and gene expression
Gurudatta Baraka Vishwanathan, Victor G. Corces.
Presenter affiliation: Emory University, Atlanta, Georgia. 16

Co-transcriptional spliceosome assembly on mammalian model genes
Nicole I. Bardehle, Karla M. Neugebauer.
Presenter affiliation: Max Planck Institute, Dresden, Germany. 17

Dynamics of histone demethylase JMJD2b
Eva Bartova, Andrea Harnicarova Horakova, Gabriela Galiova, Lenka Stixova, Stanislav Kozubek.
Presenter affiliation: Institute of Biophysics Academy of Sciences of Czech Republic, Brno, Czech Republic. 18

Chromosome repositioning correlates with changes in gene expression directed by tissue-specific nuclear envelope proteins in adipocyte differentiation
Dzmitry G. Batrakou, Gerlinde R. Otti, Jeremy Stewart, Elisabeth Wachter, Jose de las Heras, Eric C. Schirmer.
Presenter affiliation: University of Edinburgh, Edinburgh, United Kingdom. 19

Lamin A, its downstream effectors, and their prognostic value in colorectal cancer
Danielle M. Battle, Kim Smits, Robert Riedl, Manon van Engeland, Christopher J. Hutchison.
Presenter affiliation: Durham University, Durham, United Kingdom. 20

Stc1—Linking RNA interference to chromatin modification
Elizabeth H. Bayne, Sharon A. White, Alexander Kagansky, Dominika A. Bijos, Luis Sanchez-Pulido, Chris P. Ponting, Juri Rappsilber, Robin C. Allshire.
Presenter affiliation: University of Edinburgh, Edinburgh, United Kingdom. 21

Forward genetic screen for regulators of the spatial clustering of pericentric heterochromatin
David B. Beck, Roberto Bonasio, Barna Fodor, Dan Felsenfeld, Thomas Jenuwein, Danny Reinberg.
Presenter affiliation: New York University School of Medicine/ HHMI, New York, New York. 22

Identification of candidate regulators of nuclear actin by sequence analysis of human proteins
Brittany J. Belin, R. Dyche Mullins.
Presenter affiliation: UCSF, San Francisco, California. 23

The chromatin landscape of an active human kinetochore resembles the downstream region of transcribed genes
Jan H. Bergmann, Hiroshi Kimura, David Kelly, Lars E. Jansen, Hiroshi Masumoto, Vladimir Larionov, Willliam C. Earnshaw.
Presenter affiliation: University of Edinburgh, Edinburgh, United Kingdom. 24

Whole genome tumor bisulfite sequencing exposes link between nuclear architecture and aberrant DNA methylation in cancer
Benjamin P. Berman, Daniel J. Weisenberger, Joseph Aman, Toshinori Hinoue, Zachary Ramjan, Peter W. Laird.
Presenter affiliation: USC Epigenome Center, Los Angeles, California. 25

A Top2- and Hmo1-mediated architectural pathway protects the integrity of transcribed genomic regions during S-phase
Rodrigo Bermejo, Thelma Capra, Andrea Cocito, Yuki Katou, Katsuhiko Shirahige, Marco Foiani.
Presenter affiliation: FIRC Institute of Molecular Oncology Foundation, Milan, Italy. 26

The role of Condensin in centromere inheritance
Rafael Bernad, Patricia Sánchez, Teresa Rivera, Miriam Rodríguez-Corsino, Isabelle Vassias, Dominique Ray-Gallet, Geneviève Almouzni, Ana Losada.
Presenter affiliation: Spanish National Cancer Research Centre (CNIO), Madrid, Spain. 27

Loss of the histone variant macroH2A promotes melanoma progression
Emily Bernstein.
Presenter affiliation: Mount Sinai School of Medicine, New York, New York. 28

Implications for epigenetic centromere marking from the structure of the CENP-A/histone H4 heterotetramer
Nikolina Sekulic, Emily A. Bassett, Danielle J. Rogers, Ben E. Black.
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania. 29

A dynamic view of HIV-1 splicing
Stéphanie Boireau, Ute Schmidt, Eugenia Basyuk, Edouard Bertrand.
Presenter affiliation: Institut de Génétique Moléculaire, Montpellier, France. 30

Budding yeast chromosome-organizing clamps localized to the nuclear periphery by TFIIIC and Mps3
Sotirios Botsios, Anne D. Donaldson.
Presenter affiliation: University of Aberdeen, Aberdeen, United Kingdom. 31

Chromatin states and cell fate
Laurie A. Boyer, Vidya Subramanian, Lauren Surface.
Presenter affiliation: Massachusetts Institute of Technology, Cambridge, Massachusetts. 32

Cdk phosphorylation of a nucleoporin controls gene localization through the cell cycle
Donna G. Brickner, Jason H. Brickner.
Presenter affiliation: Northwestern University, Evanston, Illinois. 33

Son is essential for organization of nuclear speckles
Alok Sharma, Keshia Torres-Munoz, Michael Markey, Athanasios Bubulya, Paula A. Bubulya.
Presenter affiliation: Wright State University, Dayton, Ohio. 34

Dia2, an F-box containing protein, is involved in transcriptional silencing
Rebecca J. Burgess, Qing Li, Zhiguo Zhang.
Presenter affiliation: Mayo Clinic, Rochester, Minnesota. 35

A genetic screen to identify conditional heterochromatin mutants
Alessia Buscaino, Sharon White, Femke Simmer, Robin Allshire.
Presenter affiliation: Univerisity of Edinburgh, Edinburgh, United Kingdom. 36

Novel live cell microscopy approaches to dissect chromatin dynamics in 3D at high temporal resolution
Imen Lassadi, Houssam Hajjoul, Aurelien Bancaud, Kerstin Bystricky.
Presenter affiliation: University of Toulouse, Toulouse, France; CNRS, Toulouse, France. 37

Base excision repair within nuclear architecture—Redistribution of base excision repair proteins in response to induced DNA damage
Anna Campalans, Rachel Amouroux, Thierry Kortulewski, Bernd Epe, J. Pablo Radicella.
Presenter affiliation: CEA, Fontenay aux Roses, France. 38

Global chromatin state maps of iPS cells derived from distinct adult somatic tissues
Bryce Carey, Menno Creyghton, Mitch Guttman, Albert Cheng, Stella Markulaki, Rudolf Jaenisch.
Presenter affiliation: Whitehead Institute for Biomedical Research, Cambridge, Massachusetts; MIT, Cambridge, Massachusetts. 39

Imaging spliceosome assembly dynamics in live-cells
Teresa Carvalho, Sandra Martins, José Rino, Célia Carvalho, José Braga, Maria Carmo-Fonseca.
Presenter affiliation: Instituto de Medicina Molecular, Lisboa, Portugal. 40

Haploid plants produced through centromere-mediated genome elimination
Ravi Maruthachalam, Simon Chan.
Presenter affiliation: University of California-Davis, Davis, California. 41

Investigating the relationship between the higher-order and primary structure of chromatin in SAHFs
Tamir Chandra, Jean-Yves Thuret, Lixiang Xue, Rekin's Janky, Shamith A. Samarajiwa, Scott Newman, Masako Narita, Agustin Chicas, Paul A. Edwards, Madan M. Babu, Scott W. Lowe, Simon Tavaré, Masashi Narita.
Presenter affiliation: Cancer Research UK, Cambridge, United Kingdom. 42

Lamin A mutants target HP1 isoforms for proteasomal degradation
Pankaj Chaturvedi, Veena K. Parnaik.
Presenter affiliation: Center For Cellular and Molecular Biology, Hyderabad, India. 43

Chromatin associations with promyelocytic leukemia nuclear bodies in the interphase nucleus
Reagan W. Ching, David P. Bazett-Jones.
Presenter affiliation: The Hospital for Sick Children, Toronto, Canada; University of Toronto, Toronto, Canada. 44

Heterochromatin proteins regulate the spatial and temporal dynamics of HR repair among repeated DNAs
Irene Chiolo, Aki Minoda, Serafin U. Colmenares, Ryan Kunitake, Gary H. Karpen.
Presenter affiliation: Lawrence Berkeley National Laboratory, Berkeley, California. 45

Ndc10p—A Structural core of the inner kinetochore in budding yeast
Uhn-Soo Cho, Stephen C. Harrison.
Presenter affiliation: Harvard Medical School / HHMI, Boston, Massachusetts. 46

***De novo* assembly of a PML nuclear subcompartment induces DNA synthesis at the telomere**
Inn Chung, Karsten Rippe.
Presenter affiliation: German Cancer Research Center and BioQuant, Heidelberg, Germany. 47

Dissecting the functions of the CSR-1/22G-RNA pathway in chromosome segregation in the holocentric nematode, *C. elegans*
Julie M. Claycomb, Pedro Batista, Jie Wang, Zhiping Weng, Craig Mello.
Presenter affiliation: University of Massachusetts Medical School, Worcester, Massachusetts. 48

Modulation of the soluble pool of histone H3-H4 by the histone chaperone NASP
Adam J. Cook, Genevieve Almouzni.
Presenter affiliation: UMR218 du CNRS / Institut Curie, Paris, France. 49

Distinct functions for the two human isoforms of Anti-silencing function 1
Armelle Corpet, Leanne De Koning, Joern Toedling, Alexia Savignoni, Charlène Boumendil, Nicolas Servant, Roderick J. O'Sullivan, Jan Karlseder, Emmanuel Barillot, Bernard Asselain, Xavier Sastre-Garau, Geneviève Almouzni.
Presenter affiliation: Institut Curie, Paris, France. 50

Superresolution light microscopy of nuclear nanostructure
Christoph M. Cremer.
Presenter affiliation: University Heidelberg, Heidelberg, Germany. 51

The exon junction complex assembles into discrete nuclear regions, the perispeckles
Elisabeth Daguenet, Aurélie Baguet, Sébastien Degot, Ute Schmidt, Edouard Bertrand, Hervé Le Hir, Marie-Christine Rio, Catherine Tomasetto.
Presenter affiliation: IGBMC, Illkirch, France. 52

Long-range gene regulatory architecture of human chromosome 21
Nynke L. van Berkum, Richard Humbert, Bryan R. Lajoie, Erez Lieberman Aiden, Tobias Racogzy, Robert Thurman, Louise Williams, M.A. Bender, Eric S. Lander, Mark Groudine, John A. Stamatoyannopoulos, Job Dekker.
Presenter affiliation: University of Massachusetts Medical School, Worcester, Massachusetts. 53

Stepwise incorporation of exogenous H3.3 in mammalian cells
Erwan Delbarre, Bente-Marie Jacobsen, Anja Oldenburg, Andrew H. Reiner, Thomas M. Küntziger, Philippe Collas.
Presenter affiliation: University of Oslo, Oslo, Norway. 54

Using local photodamage to study involvement of heterochromatin protein 1 in DNA repair
Miroslaw Zarebski, Dominika Zurek, Dominika O. Trembecka, Katarzyna Kedziora, Mateusz Kuzak, Jurek W. Dobrucki.
Presenter affiliation: Jagiellonian University, Kraków, Poland. 55

Studying centromeric histone dynamics using FRET
Paul G. Donlin-Asp, Yamini Dalal.
Presenter affiliation: National Institutes of Health, Bethesda, Maryland. 56

Nucleation of nuclear bodies by RNA
Sergey P. Shevtsov, Miroslav Dundr.
Presenter affiliation: Rosalind Franklin University of Medicine and Science, North Chicago, Illinois. 57

Identification of metastable retrotransposons in the mouse genome using active histone modification marks
Muhammad B. Ekram, Keunsoo Kang, Hana Kim, Joomyeong Kim.
Presenter affiliation: Louisiana State University, Baton Rouge, Louisiana. 58

X-linked gene promoters have a different nucleosome organization than autosomal promoters in *C. elegans*
Sevinc Ercan, Yaniv Lubling, Eran Segal, Jason D. Lieb.
Presenter affiliation: University of North Carolina, Chapel Hill, North Carolina. 59

Ring1B compacts chromatin structure, and represses gene expression, independent of histone ubiquitination
Ragnhild Eskeland, Martin Leeb, Graeme Grimes, Shelagh Boyle, Anton Wutz, Wendy A. Bickmore.
Presenter affiliation: MRC Human Genetics Unit, Edinburgh, United Kingdom. 60

Infrequent associations between yeast subtelomeres are determined by chromosome arm length and nuclear constraints
Pierre Therizols, Tarn Duong, Bernard Dujon, Christophe Zimmer, Emmanuelle Fabre.
Presenter affiliation: Pasteur Institute, URA2171 CNRS, Paris, France. 61

5S rDNA pseudogenes can mediate nuclear organization and transcriptional regulation
Andrew M. Fedoriw, Joshua D. Starmer, Della Yee, Terry Magnuson.
Presenter affiliation: University of North Carolina, Chapel Hill, Chapel Hill, North Carolina. 62

p63 regulates Satb1 to control tissue-specific chromatin remodelling during development of the epidermis
Michael Y. Fessing, Andrei N. Mardaryev, Michal R. Gdula, Andrey A. Sharov, Terumi Kohwi-Shigematsu, Vladimir A. Botchkarev.
Presenter affiliation: University of Bradford, Bradford, United Kingdom. 63

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Danae Fonseca, Jorge Baquero, Frida E. Kleiman.
Presenter affiliation: Hunter College, City University of New York, New York, New York. 64

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Presenter affiliation: School of Biological Sciences, Durham, United Kingdom. 65

Heterochromatin domain fibre reorganization occurs during late stages of induced reprogramming
Eden M. Fussner, Ugljesa Djuric, Hotta Akitsu, Mike Strauss, James Ellis, David P. Bazett-Jones.
Presenter affiliation: Hospital for Sick Children, Toronto, Canada; University of Toronto, Toronto, Canada. 66

Fibronectin organizes subnuclear structure and regulates gene expression
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Presenter affiliation: National Centre for Cell Science, Pune, India; Indian Institute of Science Education and Research, Pune, India. 67

Conserved nucleosome positioning defines replication origins in yeast *S. cerevisiae*
Kiki Galani, Matthew L. Eaton, Sukhyun Kang, David M. Mac Alpine, Stephen P. Bell.
Presenter affiliation: Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts. 68

Kinetics and nuclear arrangement of Polycomb bodies
Gabriela Galiova, Stanislav Kozubek, Andrea Harnicarova Horakova, Sona Legartova, Pavel Matula, Eva Bartova.
Presenter affiliation: Institute of Biophysics Academy of Sciences of Czech Republic, Brno, Czech Republic. 69

A multifunctional cis-acting element that specifies nuclear reorganization, locus contraction and looping of immunoglobulin genes during B cell development
Yougui Xiang, Xiaorong Zhou, Susannah L. Hewitt, Jane A. Skok, William T. Garrard.
Presenter affiliation: University of Texas Southwestern Medical Center, Dallas, Texas. 70

Dissecting the human kinetochore—Chromatin interface
Karen E. Gascoigne, Iain M. Cheeseman.
Presenter affiliation: Whitehead Institute, Cambridge, Massachusetts. 71

Coordination of entry into S phase with ribosome biogenesis
Stephen M. Doris, Elizabeth Schroeder, Julia Beamesderfer, Raphyel Rosby, Susan A. Gerbi.
Presenter affiliation: Brown University, Providence, Rhode Island. 72

A mitotic histone H3 modification integrates Cdk1 and Polo-like kinase 1 activities to spatially and temporally control the activation of Aurora B
Cristina Ghenoiu, Alexander E. Kelly, John Z. Xue, Christian Zierhut, Hironori Funabiki.
Presenter affiliation: The Rockefeller University, New York, New York; Weill Cornell Graduate School of Biomedical Sciences, New York, New York. 73

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Ian D. Gibbs-Seymour, Ewa M. Markiewicz, Chris J. Hutchison.
Presenter affiliation: University of Durham, Durham, United Kingdom. 74

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Camille Godon, Pierre-Olivier Mari, Magali Philippe, Wim Vermeulen, Giuseppina Giglia-Mari.
Presenter affiliation: IPBS-CNRS-Université Paul Sabatier, Toulouse, France. 75

The role of phosphatidate phosphatase Lipin in nuclear envelope breakdown
Moritz Mall, Iain W. Mattaj, Matyas Gorjanacz.
Presenter affiliation: European Molecular Biology Laboratory, Heidelberg, Germany. 76

Acute prelamin A accumulation leads to alterations in nuclear morphology and function
Chris N. Goulbourne, Ashraf N. Malhas, David J. Vaux.
Presenter affiliation: University of Oxford, Oxford, United Kingdom. 77

Promoter architecture and susceptibility to RNA-induced transcriptional gene silencing
Victoria A. Green, Marc S. Weinberg, Patrick Arbuthnot.
Presenter affiliation: Antiviral Gene Therapy Research Unit, Johannesburg, South Africa. 78

Imaging transcription initiation of endogenous genes in live cells
Benjamin Guglielmi, Robert Tjian.
Presenter affiliation: University of California-Berkeley, Berkeley, California. 79

H3 lysine 4 acetylation regulates gene expression and is confined to promoters by H3 lysine 4 tri-methylation
Benoit Guillemette, Paul Drogaris, Hsiu-Hsu Sophia Lin, Axel Imhof, Pierre Thibault, Alain Verreault, Richard J. Festenstein.
Presenter affiliation: Imperial College London, London, United Kingdom; University of Montreal, Montreal, Canada. 80

RPA-dependent recruitment of human DNA helicase B to stalled replication forks
Gulfem D. Guler, Hanjian Liu, Ellen Fanning.
Presenter affiliation: Vanderbilt University, Nashville, Tennessee. 81

Lack of canonical histone synthesis causes cell cycle arrest after DNA replication
Ufuk Günesdogan, Herbert Jäckle, Alf Herzig.
Presenter affiliation: Max Planck Institute for Biophysical Chemistry, Göttingen, Germany. 82

Interactions between the DNA damage and spindle checkpoints in budding yeast
James E. Haber, Satoshi Yoshida, Dotiwala Farokh, Neal Sugawara, Jacob Harrison.
Presenter affiliation: Brandeis University, Waltham, Massachusetts. 83

Heterochromatin dynamics—A network of mobile and stable proteins forms a major repressive chromatin domain
Matthias Hahn, Lothar Schermelleh, Gunnar Schotta.
Presenter affiliation: Ludwig-Maximilians-Universität München, Munich, Germany. 84

Features of spatial nuclear organization in dynamic gene expression
Ofir Hakim, Myong-Hee Sung, Ty C. Voss, Erik Splinter, Sam John, Wouter de Laat, Gordon L. Hager.
Presenter affiliation: National Institute of Health, Bethesda, Maryland. 85

Biased distribution of the nuclear pore complex contributes to nuclear differentiation in ciliate *Tetrahymena*
Tokuko Haraguchi, Masaaki Iwamoto, Fumihide Bunai, Chie Mori, Tomoko Kojidani, Hiroko Osakada, Takako Koujin, Haruhiko Asakawa, Yasushi Hiraoka.
Presenter affiliation: NICT, Kobe, Japan; Osaka University, Toyonaka, Japan; Osaka University, Suita, Japan. 86

TFIIH kinase is a general regulator of RNA Polymerase II transcription but regulates capping only on specific genes in mammalian cells
Katja Helenius, Ying Yang, Timofey V. Tselykh, Heli K. Pessa, Mikko J. Frilander, Tomi P. Mäkelä.
Presenter affiliation: University of Helsinki, Helsinki, Finland. 87

Nuclear structural networks: Lamin A/C provides hardware for nuclear lamina construction and regulation of gene expression
Harald Herrmann, Dorothee Möller, Norbert Mücke, Larisa E. Kapinos, Aebi Ueli.
Presenter affiliation: German Cancer Research Center, Heidelberg, Germany. 88

THURSDAY, June 3—4:30 PM

Wine and Cheese Party

THURSDAY, June 3—7:30 PM

SESSION 4 DNA REPLICATION AND GENOME INTEGRITY

Chairperson: **K. Nasmyth,** University of Oxford, United Kingdom

Mechanism and regulation of DNA replication during the cell cycle and in response to DNA damage
John Diffley.
Presenter affiliation: Cancer Research UK London Research Institute, South Mimms, United Kingdom. 89

Space and time in the nucleus—Developmental control of replication timing and chromosome architecture
Ichiro Hiratani, Tyrone Ryba, David M. Gilbert.
Presenter affiliation: Florida State University, Tallahassee, Florida. 90

Epigenetic challenges in centromere inheritance during the cell cycle
Geneviève Almouzni.
Presenter affiliation: CNRS UMR 218 / Institut Curie, Paris, France. 91

The Origin Recognition Complex (ORC) is required for chromosome segregation during mitosis
Bruce Stillman, Shuang Ni, Kipp Weiskopf, Supriya Prasanth.
Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 92

How mammalian telomeres solve the end-protection problem
Titia de Lange.
Presenter affiliation: The Rockefeller University, New York, New York. 93

The SMC5/6 complex—Resolving topological tension during chromosome replication?
Andreas Kegel, Hanna B. Lindroos, Takaharu Kanno, Kristian Jeppsson, Lena Ström, Takehiko Itoh, Katsuhiko Shirahige, Camilla Sjögren.
Presenter affiliation: Karolinska Institutet, Stockholm, Sweden. 94

Global regulation of H2A.Z localization by the INO80 chromatin remodeling complex is essential for genome integrity
Manolis Papamichos-Chronakis, Shinya Watanabe, Oliver Rando, Craig L. Peterson.
Presenter affiliation: University of Massachusetts Medical School, Worcester, Massachusetts. 95

FRIDAY, June 4—9:00 AM

SESSION 5 REPROGRAMMING AND DIFFERENTIATION

Chairperson: **T. de Lange,** Rockefeller University, New York, New York

Molecular mechanisms of pluripotency and reprogramming
Rudolf Jaenisch.
Presenter affiliation: Whitehead Institute for Biomedical Research, Cambridge, Massachusetts. 96

Resetting the mouse epigenome in germ cells for totipotency
Azim Surani, Petra Hajkova.
Presenter affiliation: University of Cambridge, Cambridge, United Kingdom. 97

Establishing genetic competence for chromatin in development and mitosis
Ken Zaret, Juanma Caravaca, Masashi Abe, Cheng-Ran Xu.
Presenter affiliation: University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania. 98

Connecting transcriptional control to chromosome structure and human disease
Richard A. Young.
Presenter affiliation: Whitehead Institute and MIT, Cambridge, Massachusetts. 99

Deciphering and reconstructing the embryonic stem cell transcriptional regulatory network
Huck H. Ng.
Presenter affiliation: Genome Institute of Singapore, Singapore, Singapore. 100

Changes in chromatin fibre density as a marker for pluripotency
David P. Bazett-Jones, Eden Fussner, Kashif Ahmed, Hesam Dehghani, Peter Rugg-Gunn, Ugljesa Djuric, Akitsu Hotta, Janet Rossant, James Ellis.
Presenter affiliation: Hospital for Sick Children, Toronto, Canada. 101

***In vitro* reprogramming of somatic cells to cancer stem cells**
Paola Scaffidi, Tom Misteli.
Presenter affiliation: National Cancer Institute, Bethesda, Maryland. 102

FRIDAY, June 4—2:00 PM

SESSION 6 POSTER SESSION II

Cytoplasmic tail of EGFR ligand, amphiregulin regulates histone modification and induces the cell migration
Hisae Tanaka, Miki Hieda, Yu Nishioka, Shigeki Higashiyama, Nariaki Matsuura.
Presenter affiliation: Osaka University Graduate School of Medicine, Suita, Japan. 103

RanGAP1-regulated cytoplasmic diffusion of nuclear proteins during meiosis in fission yeast
Haruhiko Asakawa, Tomoko Kojidani, Chie Mori, Hiroko Osakada, Tokuko Haraguchi, Yasushi Hiraoka.
Presenter affiliation: Osaka University, Suita, Japan; NICT, Kobe, Japan; Osaka University, Toyonaka, Japan. 104

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Presenter affiliation: The Whitehead Institute, Cambridge, Massachusetts. 105

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Presenter affiliation: University of Chicago, Chicago, Illinois. 106

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Presenter affiliation: University of California San Francisco, San Francisco, California; University of Edinburgh, Edinburgh, United Kingdom. 107

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Presenter affiliation: Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic. 108

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Presenter affiliation: University of Cambridge, Cambridge, United Kingdom. 109

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Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 110

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Presenter affiliation: Max-Planck Institute of Immunobiology, Freiburg, Germany; European Molecular Biology Laboratory, Heidelberg, Germany. 111

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Presenter affiliation: Osaka Medical Center for Cancer, Osaka, Japan. 112

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Presenter affiliation: University of Florida College of Medicine, Gainesville, Florida. 113

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Presenter affiliation: The Wistar Institute, Philadelphia, Pennsylvania. 114

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Presenter affiliation: UNAM, Universidad Nacional Autónoma de México, Mexico City, Mexico. 115

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Presenter affiliation: Institute of Molecular Genetics, Montpellier, France. 116

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Presenter affiliation: Academia Sinica, Taipei, Taiwan. 118

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Presenter affiliation: University of Virginia, Charlottesville, Virginia. 119

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Presenter affiliation: Louisiana State University, Baton Rouge, Louisiana. 123

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Presenter affiliation: Netherlands Cancer Institute, Amsterdam, The Netherlands. 126

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Presenter affiliation: University of Toronto, Toronto, Ontario, Canada; Ontario Cancer Institute, Toronto, Ontario, Canada. 132

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Presenter affiliation: Wellcome Trust Centre for Cell Biology, Edinburgh, United Kingdom. 134

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Presenter affiliation: University of California, Berkeley, Berkeley, California. 135

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Presenter affiliation: Northwestern University Feinberg School of Medicine, Chicago, Illinois. 136

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Presenter affiliation: Harvard-MIT, Cambridge, Massachusetts. 137

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Presenter affiliation: Northwestern University, Evanston, Illinois. 138

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Presenter affiliation: MRC Human Genetics Unit, Edinburgh, United Kingdom. 139

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Presenter affiliation: Institut de Genetique Moleculaire de Montpellier, Montpellier, France. 140

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Presenter affiliation: National Institute of Genetics, Mishima, Japan; RIKEN, Sayo-cho, Japan; RIKEN, Wako, Japan. 141

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Presenter affiliation: Cornell University, Ithaca, New York. 142

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Presenter affiliation: University of Edinburgh, Edinburgh, United Kingdom. 143

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Presenter affiliation: CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), Toulouse, France. 144

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Presenter affiliation: Biocenter Ludwig Maximilians University (LMU) Munich, Martinsried, Germany. 145

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Presenter affiliation: Instituto de Medicina Molecular (IMM), Lisbon, Portugal. 146

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Presenter affiliation: Hokkaido University, Sapporo, Japan. 147

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Presenter affiliation: Curie Institute, Paris, France. 148

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Rachel P. McCord, Vicky Zhou, Tiffany Yuh, Martha L. Bulyk.
Presenter affiliation: Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts; Harvard University, Boston, Massachusetts. 149

Cancer diagnostics based on interphase spatial genome positioning
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Presenter affiliation: National Cancer Institute/NIH, Bethesda, Maryland. 150

Dynamics of single mRNP nucleo-cytoplasmic transport through the nuclear pore in living cells
Amir Mor, Shimrit Suliman, Rakefet Ben-Yishay, Sharon Yunger, Yehuda Brody, Yaron Shav-Tal.
Presenter affiliation: Bar-Ilan University, Ramat Gan, Israel. 151

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Presenter affiliation: IPBS, Toulouse, France. 152

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Presenter affiliation: Arizona State University, Tempe, Arizona. 154

CTCF is critical for higher order folding of the β-globin domain
Ye Zhan, Natalia Naumova, Bryan Lajoie, Marc A. Marti-Renom, Davide Bau, Job Dekker.
Presenter affiliation: University of Massachusetts Medical School, Worcester, Massachusetts. 155

A mechanism linking whole-chromosome aneuploidy to DNA breaks
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Presenter affiliation: Dana-Farber Cancer Institute, Howard Hughes Medical Institute, Boston, Massachusetts. 156

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Presenter affiliation: Genome Institute of Singapore, Singapore, Singapore. 157

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Presenter affiliation: Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland. 158

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Presenter affiliation: Institut Curie Inserm/CNRS U934/UMR3215, Paris, France. 159

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Presenter affiliation: The Rockefeller University, New York, New York. 160

POGZ modulates HP1 dissociation from mitotic chromosome arms for correct activation of Aurora B kinase in human cells
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Presenter affiliation: Hokkaido University Graduate School of Life Science, Sapporo, Japan. 161

Identification of a *cis*-element that regulates the association of cohesin with chromosomes
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Presenter affiliation: Howard Hughes Medical Institute, Carnegie Institution, Baltimore, Maryland. 162

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Presenter affiliation: Boston University School of Medicine, Boston, Massachusetts. 164

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Presenter affiliation: University of Leicester, Leicester, United Kingdom. 165

An actin-regulated importin α/β-dependent extended bipartite NLS directs nuclear import of MRTF-A
Rafal Pawlowski, Eeva K. Rajakylä, Maria K. Vartiainen, Richard Treisman.
Presenter affiliation: Cancer Research UK, London Research Institute, London, United Kingdom. 166

Mechanism of repression of oligomeric oncogenic transcription factor fusions
Norihiko Kawamata, Mario A. Pennella, Jennifer L. Woo, Arnold J. Berk, Harold P. Koeffler.
Presenter affiliation: UCLA, Los Angeles, California. 167

Wapl and cohesin control higher order chromatin structure in interphase chromosomes
Antonio Tedeschi, Gordana Wutz, Venugopal Bhaskara, Jan-Michael Peters.
Presenter affiliation: Research Institute of Molecular Pathology, Vienna, Austria. 168

Chromatin organization and –dynamics in mouse stem cells and induced pluripotent stem cells
Katrin S. Pfleghaar, Stephanie Peklo, Hilmar Strickfaden, Andreas Zunhammer, Gustavo Folle, Thomas Cremer.
Presenter affiliation: LMU Muenchen Biozentrum, Martinsried, Germany. 169

What sets the size-limit of a nuclear body? Insights from localization of various portions of the long noncoding RNA, NEAT1, by high resolution *in situ* hybridization within paraspeckles
Sylvie Souquere, Guillaume Beauclair, Francis Harper, Archa Fox, Gérard Pierron.
Presenter affiliation: CNRS FRE-3238, Villejuif, France. 170

Nuclear-retained non-coding RNA regulates alternative splicing by modulating SR splicing factor phosphorylation
Vidisha Tripathi, Jonathan Ellis, Zhen Shen, David Song, Susan M. Freier, C. Frank Bennett, Alok Sharma, Paula A. Bubulya, Benjamin J. Blencowe, Supriya G. Prasanth, Kannanganattu V. Prasanth.
Presenter affiliation: University of Illinois at Urbana-Champaign, Urbana, Illinois. 171

Histone pre-mRNA processing defects disrupt coilin localization in *Drosophila*
Kavita Praveen, A.Gregory Matera.
Presenter affiliation: University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. 172

A chromatin insulator imposes early replication timing
Vahideh Hassan-Zadeh, Sabaranidh Chilaka, Jean-Charles Cadoret, Nicole Bogetto, Adam West, Marie-Noëlle Prioleau.
Presenter affiliation: CNRS, Université Paris 7, Paris, France. 173

Genome-wide methylation profiling for the identification of new imprinted genes
Charlotte Proudhon, Reiner Schulz, Rebecca Oakey, Vardhman Rakyan, Déborah Bourc'his.
Presenter affiliation: Institut Curie, Paris, France. 174

Regulation of mRNA export by the phosphatidylinositol 3 kinase/AKT pathway
Alexandre J. Quaresma, Rachel Sievert, Jeffrey A. Nickerson.
Presenter affiliation: University of Massachusetts Medical School, Worcester, Massachusetts. 175

Networking the nucleus—Does form precede or follow the function?
Indika Rajapakse, David Scalzo, Stephen Tapscott, Mark Groudine.
Presenter affiliation: Fred Hutchinson Cancer Research Center, Seattle, Washington. 176

Drosophila topoisomerase II association with chromatin is delimited by insulators and demarcates genome-wide transcription and replication
Edward Ramos, Eduardo A. Torre, Victor G. Corces.
Presenter affiliation: Emory University, Atlanta, Georgia. 177

The PML NB-dependent localization of Daxx during myogenesis
Lindsy M. Rapkin, David P. Bazett-Jones.
Presenter affiliation: The Hospital for Sick Children, Toronto, Canada; University of Toronto, Toronto, Canada. 178

A fraction of MCM 2-7 proteins remains associated with replication foci during a major part of S-phase
Ivan Raska.
Presenter affiliation: Charles University in Prague, Prague, Czech Republic; Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic. 179

FRIDAY, June 4—7:00 PM

Reginald B. Harris Lecture

Transcription, RNA processing, and nuclear bodies
Zehra Nizami, Svetlana Deryusheva, Joseph G. Gall.
Presenter affiliation: Carnegie Institution for Science, Baltimore, Maryland. 180

SESSION 7 RNA BIOLOGY

Chairperson: **D. Spector,** Cold Spring Harbor Laboratory, New York, New York

Downregulation of a host microRNA by a viral noncoding RNA
Demain Cazalla, Therese Yario, Joan A. Steitz.
Presenter affiliation: Howard Hughes Medical Institute, Yale University, New Haven, Connecticut. 181

Long non-coding RNAs with enhancer-like functions in human
Ulf Andersson Ørom, Ramin Shiekhattar.
Presenter affiliation: The Wistar Institute, Philadelphia, Pennsylvania. 182

Nuclear dynamics of X-chromosome inactivation
Edith Heard.
Presenter affiliation: CNRS UMR 218, Institut Curie, Paris, France. 183

Pinning down non-coding RNAs in chromosome regulation
Jeanne B. Lawrence, Alvin V. Gomez, Lisa L. Hall.
Presenter affiliation: University of Massachusetts Medical School, Worcester, Massachusetts. 184

The perinucleolar compartment associates with novel RNA-protein interactions
Callie Pollock, Kelly Daily, Chen Wang, Sui Huang.
Presenter affiliation: Northwestern Unversity, Feinberg School of Medicine, Chicago, Illinois. 185

SATURDAY, June 5—9:00 AM

SESSION 8 CHROMOSOME STRUCTURE AND MITOSIS

Chairperson: **A. Amon,** Massachusetts Institute of Technology, Cambridge

Multiple steps leading to the establishment of sister chromatid cohesion
Kim Nasmyth.
Presenter affiliation: University of Oxford, Oxford, United Kingdom. 186

Condensin and microtubule tension drive de-catenation by topoisomerase II through stable modification of DNA topology
Jonathan Baxter, Jorge Schvartzman, John Diffley, Luis Aragon.
Presenter affiliation: Medical Research Council, London, United Kingdom. 187

Structural studies of the budding-yeast kinetochore
Stephen C. Harrison, Uhn-soo Cho, Kevin Corbett, John Bellizzi, Kim Simons, Peter K. Sorger.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 188

The epigenetic basis of centromere identity and maintenance
Don W. Cleveland, Daniel Foltz, Lars Jansen, Ben Black.
Presenter affiliation: Ludwig Institute for Cancer Research/UCSD, La Jolla, California. 189

Interplay of kinetochore geometry and tension determines chromosome orientation during mitosis and meiosis
Yoshinori Watanabe.
Presenter affiliation: University of Tokyo, Yayoi, Tokyo, Japan. 190

How centrosome duplication occurs exactly once per cell cycle
Emily Hatch, Tim Stearns.
Presenter affiliation: Stanford University, Stanford, California. 191

Using multi-dimensional proteomics to define the complete protein composition of mitotic chromosomes
Shinya Ohta, Jimi-Carlo Bukowski-Wills, Laura C. Wood, Juri Rappsilber, William C. Earnshaw.
Presenter affiliation: University of Edinburgh, Edinburgh, United Kingdom. 192

SATURDAY, June 5—2:00 PM

SESSION 9 HETEROCHROMATIN FORMATION AND GENE SILENCING

Chairperson: **G. Almouzni,** CNRS UMR218, Institut Curie, Paris, France

Epigenetic genome control by heterochromatin and RNAi machinery
Shiv I. Grewal.
Presenter affiliation: Laboratory of Biochemistry and Molecular Biology, Bethesda, Maryland. 193

Making CENs of heterochromatin
Robin C. Allshire.
Presenter affiliation: Wellcome Trust Centre for Cell Biology, Edinburgh, United Kingdom. 194

Roles of RNA polymerases IV and V in siRNA-mediated chromatin modification and interphase chromatin organization
Olga Pontes, Alexa Vitins, Jeremy R. Haag, Pedro Costa-Nunes, Craig S. Pikaard.
Presenter affiliation: Indiana University, Bloomington, Indiana. 195

Gene punctuation—Multiple roles of transcriptional termination in regulating eukaryotic gene expression
Nicholas J. Proudfoot, Jurgi Camblong, Monika Gullerova, Sue Mei Tan Wong, Hashanthi Wijayatilake.
Presenter affiliation: University of Oxford, Oxford, United Kingdom. 196

Consequences of aneuploidy
Angelika Amon.
Presenter affiliation: Howard Hughes Medical Institute/Massachusetts Institute of Technology, Cambridge, Massachusetts. 197

SATURDAY, June 5—5:30 PM

DORCAS CUMMINGS LECTURE

Robert Goldman
Northwestern University Medical School

”The Unexpected Link Between Premature Aging and Nuclear Organization”

SUNDAY, June 6—9:00 AM

SESSION 10 TRANSCRIPTION MEETS RNA PROCESSING

Chairperson: **J. Steitz,** Howard Hughes Medical Institute, Yale University, New Haven, Connecticut

Connecting transcription with mRNA processing and chromatin modifications
Stephen Buratowski.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 198

Chromatin, pol II elongation and alternative splicing
Mariano Allo, Manuel J. Muñoz, Ignacio E. Schor, Alberto R. Kornblihtt.
Presenter affiliation: Universidad de Buenos Aires and CONICET, Buenos Aires, Argentina. 199

Histone H3 lysine 9 tri-methylation and HP1γ favor inclusion of alternative exons
Violaine Saint André, Eric Batsché, Christophe Rachez, Christian Muchardt.
Presenter affiliation: Institut Pasteur, URA2578 du CNRS, Paris, France. 200

Connecting the genome to the cytoplasm—The view from genetic screens to the single cell
Pamela A. Silver, William Senapedis, Michael Moore, Zeev Waks.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 201

Genetic analysis of nuclear body function
A. Gregory Matera, T.K. Rajendra, Kavita Praveen.
Presenter affiliation: University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. 202

Following wingle mRNAs from synthesis to decay in living cells
Robert H. Singer.
Presenter affiliation: Albert Einstein College of Medicine, Bronx, New York. 203

mRNP rearrangements during the pioneer round of translation, nonsense-mediated mRNA decay, and thereafter
Jungwook Hwang, Hanae Sato, Chenguang Gong, Yalan Tang, Lynne E. Maquat.
Presenter affiliation: University of Rochester, Rochester, New York. 204

SUNDAY, June 6—2:00 PM

SESSION 11 POSTER SESSION III

Human ISWI chromatin remodeling complexes identify their nucleosome substrates via a continuous sampling mechanism
Fabian Erdel, Thomas Schubert, Gernot Längst, Karsten Rippe.
Presenter affiliation: German Cancer Research Center, Heidelberg, Germany. 205

Swi1, a component of the fork protection complex, protects replisome components against degradation
Laura C. Roseaulin, Eishi Noguchi.
Presenter affiliation: Drexel University College of Medecine, Philadelphia, Pennsylvania. 206

Opposing effects of nucleolar oncoproteins on rRNA transcription
Stefano Rossetti, Andre T. Hoogeveen, Nicoletta Sacchi.
Presenter affiliation: Roswell Park Cancer Institute, Buffalo, New York. 207

The SUN domain contributes to nuclear envelope targeting of human SUN2
Andrea Rothballer, Rosemarie Ungricht, Yagmur G. Turgay, Ulrike Kutay.
Presenter affiliation: ETH Zurich, Zurich, Switzerland. 208

Novel constituents of the outer nuclear membrane
Dae In Kim, Laura Fishwick, Steve C. Tovey, Henning Horn, Colin W. Taylor, Colin L. Stewart, Brian Burke, Kyle J. Roux.
Presenter affiliation: University of Florida, Gainesville, Florida. 209

The Bloom's syndrome protein is essential for the centromeric disjunction process
Sébastien Rouzeau, Fabrice P. Cordelières, Géraldine Buhagiar-Labarchède, Kenza Lahkim Bennani-Belhaj, Ilse Hurbain, Rosine Onclercq-Delic, Laura Magnaghi-Jaulin, Christian Jaulin, Mounira Amor-Guéret.
Presenter affiliation: Institut Curie, Orsay, France. 210

Inheritance of histone acetylation and H3K9 methylation through S-phase is regulated by ATP-dependent chromatin remodeller SMARCAD1
Samuel P. Rowbotham, Jacqueline E. Mermoud, Leila Bakri-Celli, Patrick Varga-Weisz.
Presenter affiliation: Babraham Institute, Cambridge, United Kingdom. 211

Imaging single protein molecules in live eukaryotic cell nucleus
Rahul Roy, Srinjan Basu, Gene-Wei Li, Sunney X. Xie.
Presenter affiliation: Harvard University, Cambridge, Massachusetts. 212

RARB2 chromatin inability to transition to the poised state is a common denominator of silent and active RARB2 in cancer cells
Nicoletta Sacchi, Stefano Rossetti.
Presenter affiliation: Roswell Park Cancer Institute, Buffalo, New York. 213

Novel link between nuclear speckles formation and Ran-RanBP2 system
Noriko Saitoh, Mitsuyoshi Nakao.
Presenter affiliation: Kumamoto University, Kumamoto, Japan. 214

Live-cell analyses reveal how multiple replicons are processed for replication at individual factories
Nazan Saner, Toyoaki Natsume, Marek Gierlinski, Julian J. Blow, Tomoyuki U. Tanaka.
Presenter affiliation: University of Dundee, College of Life Sciences, Dundee, United Kingdom. 215

Formation of a cell cycle dependent dynamic HMGA1a/ORC/HP1alpha complex in heterochromatin
Andreas W. Thomae, Benjamin Vogel, Jens Baltin, Manuel J. Deutsch, Robert Hock, Aloys Schepers.
Presenter affiliation: Helmholtz Zentrum München, München, Germany. 216

Spatio-temporal organization of DNA replication structures at super-resolution with 3-dimensional structured illumination microscopy (3D-SIM)
Lothar Schermelleh, Vadim O. Chagin, Heinrich Leonhardt, M Cristina Cardoso.
Presenter affiliation: Ludwig Maximilians University Munich, Planegg-Martinsried, Germany. 217

Tissue-specific nuclear envelope proteins can redirect specific chromosomes to the nuclear periphery
Nikolaj Zuleger, Shelagh Boyle, Dzmitry Batrakou, Jose de las Heras, Wendy Bickmore, Eric Schirmer.
Presenter affiliation: University of Edinburgh, Edinburgh, United Kingdom. 218

The interplay of DNA supercoiling and catenation during the segregation of sister duplexes—Prokaryotes vs. Eukaryotes
Virginia López, Estefanía Monturus, Jonathan Baxter, Guillaume Witz, Andrzej Stasiak, María-Luisa Martínez-Robles, Pablo Hernández, Dora B. Krimer, John F X. Diffley, Luis Aragon, Jorge B. Schvartzman.
Presenter affiliation: Centro de Investigaciones Biológicas (CSIC), Madrid, Spain. 219

Pairing of homologous chromosome regions correlates with their frequency of mitotic recombination
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Presenter affiliation: Stowers Institute for Medical Research, Kansas City, Missouri. 229

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Presenter affiliation: Fred Hutchinson Cancer Research Center, Seattle, Washington; University of Washington, Seattle, Washington. 257

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Presenter affiliation: MRC Cancer Cell Unit, Cambridge, United Kingdom; MRC Laboratory of Molecular Biology, Cambridge, United Kingdom. 258

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Presenter affiliation: Massachusetts General Hospital, Boston, Massachusetts. 260

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Presenter affiliation: Howard Hughes Medical Institute, University of California-Berkeley, Berkeley, California; King's College London, London, United Kingdom. 261

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Presenter affiliation: Stony Brook University, Stony Brook, New York; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 268

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Jianmei Zhu, Karen J. Ouyang, Nathan A. Ellis, Michael J. Matunis.
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SUNDAY, June 6—4:00 PM

BEACH PICNIC

SUNDAY, June 6—7:00 PM

SESSION 12 NUCLEAR PERIPHERY

Chairperson: **W. Earnshaw,** University of Edinburgh, United Kingdom

Dynamics of genome–nuclear lamina interactions
Bas van Steensel.
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The nuclear lamins—Building blocks of nuclear architecture
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Presenter affiliation: Northwestern University, Chicago, Illinois. 271

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Presenter affiliation: Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland. 273

The architecture and transport mechanism of the nuclear pore complex
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Christine Doucet, Jessica Talamas, Martin W. Hetzer.
Presenter affiliation: Salk Institute for Biological Studies, La Jolla, California. 275

MONDAY, June 7—9:00 AM

SESSION 13 CHROMATIN STRUCTURE AND ORGANIZATION

Chairperson: **S. Gasser,** Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

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Sara Sandin, Andrew Routh, Daniela Rhodes.
Presenter affiliation: MRC Laboratory of Molecular Biology, Cambridge, United Kingdom. 276

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Presenter affiliation: University of Illinois, Urbana-Champaign, Urbana, Illinois. 277

Chromosome territories and the interchromatin compartment—A functional marriage
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The transcriptional interactome, preferential spatial organization of co-regulated genes
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Presenter affiliation: The Babraham Institute, Cambridge, United Kingdom. 280

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Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 281

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Amanda Fisher.
Presenter affiliation: Imperial College School of Medicine, London, United Kingdom. 282

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SESSION 14 GENOME REGULATION

Chairperson: **E. Heard,** CNRS UMR128, Institut Curie, Paris, France

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Presenter affiliation: German Cancer Research Center, Heidelberg, Germany. 286

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Presenter affiliation: The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland. 287

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Steven Henikoff
Fred Hutchinson Cancer Research Center

MONDAY, June 7—6:00 PM

BANQUET

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NUCLEAR REPROGRAMMING BY EGGS AND OOCYTES.

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As cells differentiate they become increasingly committed to their final specialization. However, the stability of their differentiated state can be experimentally reversed by procedures that include nuclear transfer, cell fusion, and iPS technology. This talk will discuss chromatin changes associated with nuclear transfer to eggs and oocytes. When trying to analyze the mechanisms responsible for nuclear reprogramming and gene activation, it is helpful to have an experimental system in which the reprogramming takes place rapidly and efficiently. If the efficiency of the nuclear reprogramming is judged by the ability of nuclei of cells from one specialized path of differentiation to generate cells of another unrelated pathway, then the efficiency of reprogramming by eggs in second meiotic metaphase is about 30%. However, the events that immediately follow the transplantation of somatic nuclei to eggs are difficult to analyze because, immediately after nuclear transfer to eggs, the major activity of a transplanted nucleus is to replicate DNA, and transcription starts only after many hours (mammals) or many cell divisions (amphibia). When somatic nuclei are transplanted to the germinal vesicle of an oocyte in first meiotic prophase, a very efficient activation of previously quiescent pluripotency genes takes place. This experimental system is being used to identify the components of oocytes that induce pluripotency gene reactivation and which elicit major changes to the structure and composition of chromatin. An important and necessary part of the reprogramming process is the substitution of the somatic linker histone H1o by the oocyte or embryo-specific linker histone B4 H1foo. This is a necessary change to the chromatin without which gene reactivation does not take place. When chromatin has become decondensed by action of linker histone exchange and other substitutions, the genome of transplanted nuclei appears to be able to respond to basic transcriptional factors and not to require gene-specific transcription factors. The combination of structural chromatin changes and an insensitivity to gene-specific transcription factors begins to approach a mechanistic explanation for the reprogramming of transplanted nuclei by first meiotic prophase oocytes. It will be argued that the same processes prepare the second meiotic metaphase egg for rapid response to fertilization and for the immediate initiation of transcription, followed by the lineage-dependent selection of gene activities.

COPYING AND REPROGRAMMING OF HETEROCHROMATIN WITH RNAI

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Heterochromatin is composed of transposable elements (TE) and related repeats which silence genes located nearby, and play a major role in epigenetic regulation of the genome. Far from being inert, heterochromatin is transcribed and small interfering RNA corresponding to heterochromatic sequences can be detected in plants, animals and fission yeast. In plants, small interfering RNA (siRNA) corresponding to some classes of TE depends on DNA methyltransferase MET1, the SWI/SNF ATPase, DDM1, or both, but not on the histone deacetylase SIL1/HDA6. All three genes are required for silencing transposons in the absence of siRNA. Down regulation of DDM1 and MET1 in pollen companion cells leads to heterochromatin reprogramming, and the translocation of siRNA into the sperm. In ovules, a similar mechanism silences transposons in female gametes but also appears to inhibit the differentiation of unreduced asexual gametes.

In fission yeast and in Arabidopsis, centromeric repeats are transcribed, but the transcripts are rapidly turned over by RNA interference, through the combined action of DNA dependent RNA polymerase, Argonaute and RNA dependent RNA polymerase, each of which is associated with heterochromatin. Histone H3 lysine-9 dimethylation (H3K9me2) depends on RNAi, mediated by the Rik1-Clr4 complex. We have found that heterochromatin is lost transiently during chromosomal replication, allowing heterochromatic transcripts to accumulate. Rapid processing of these transcripts into small RNA during S phase promotes restoration of heterochromatic modifications and the retention of cohesin in G2. These results explain how “silent” heterochromatin can be transcribed, and lead to a model for epigenetic inheritance during replication.

TARGETING X CHROMOSOMES FOR REPRESSION

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Gene expression in metazoans is controlled by diverse regulatory mechanisms that function over a wide range of distances. Some mechanisms affect expression of genes across large chromosomal territories or along entire chromosomes, while others only act locally on individual genes. In the former case, specific chromosome architecture and chromosome compartmentalization within the nucleus can be critical for controlling gene expression. X-chromosome dosage compensation is exemplary for dissecting the coordinate regulation of gene expression over vast distances and the role of chromosome structure in controlling gene expression. Dosage compensation ensures that males (XO or XY) and females (XX) express equal levels of gene products along X, despite their difference in X-chromosome dose. Strategies for dosage compensation differ, but invariably a regulatory complex is targeted to the sex chromosome of one sex to modulate transcript levels across the entire chromosome. In the nematode C. elegans, a specialized dosage compensation complex (DCC) is targeted to both X chromosomes of hermaphrodites to repress transcript levels by about half. The DCC resembles condensin, a protein complex required for the compaction, resolution, and segregation of chromosomes during both mitosis and meiosis. We have shown the DCC not only resembles condensin, it shares components with two other condensin complexes in the worm, and DCC components also participate in other aspects of chromosome dynamics, including chromosome segregation and the regulation of crossover number and distribution during meiosis. This talk will focus on mechanisms by which the DCC recognizes and binds X chromosomes, the mode of DCC action, and the roles of protein modification and nuclear pore complexes in the binding and functioning of the DCC.

THE NUCLEAR ENVELOPE AND CHROMATIN ORGANIZATION

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The nuclear envelope (NE) is an evolutionary derivative of the prokaryotic plasma membrane. It evolved to segregate chromatids and to assist in its three-dimensional organization. The NE's principal landmarks are the huge nuclear pore complexes (NPCs) and the recently discovered NE junctions (NEJs).

NPCs likely arose from protein modules that stabilize sharp membrane bends. Further acquisition of other proteins transformed these modules into the present-day complex organelles for bi-directional transport between the nucleus and the cytoplasm. These transport organelles are unique for being able to handle a huge size range of transport substrates. Subjacent to NPCs are less compacted regions (euchromatin) of chromatids.

The NEJs consist of patches of closely associated integral membrane proteins of the inner and outer NE membrane. These patches are linked in the peri-nuclear space through conserved peri-nuclear domains. The diverse cytoplasmic domains of NEJ proteins are joined to cytoplasmic filaments (actin filaments, intermediate filaments, microtubules), whereas the nucleoplasmic domains are indirectly associated with compacted regions (heterochromatin) of chromatids. The links between heterochromatin, NEJs and cytoskeleton transmit mechanical forces that are generated by the cytoskeleton to chromatids. This generates nuclear zones of random compression and de-compression. These zones would facilitate the large range of chromatid conformations that occur in transcription, DNA repair and replication.

NEW VIEWS OF LOCAL AND GENOME-WIDE TRANSCRIPTION ACTIVATION IN VIVO

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Many gene promoters have distinctive architectural features that allow them to be rapidly and synchronously responsive to regulatory signals. Using both gene-focused and genome-wide approaches, we are assessing the mechanisms of gene activation in Drosophila and mammalian systems. Our gene-focused studies in Drosophila examine by both optical and biochemical approaches the mechanisms of activation of heat shock genes and the accompanying dramatic changes in chromatin structure in vivo. Hsp70 provides a well-characterized example of promoter-proximal paused RNA polymerase II (Pol II) that we are using to investigate the role of specific factors and sequence elements in establishing the paused Pol II and in modulating its escape into productive elongation upon heat shock activation.

We are also examining the rapid and transcription-independent propagation of nucleosome loss throughout the heat shock loci. This nucleosome loss is dependent on the master regulatory protein, HSF, and Poly(ADP)-Ribose polymerase (PARP). PARP resides in a peak centered on the first nucleosomes at the 5' end of the Hsp70 gene prior to heat shock, and is lost from the 5’ end immediately following heat shock and redistributes downstream and halting near insulating elements surrounding the Hsp70 locus. This rapid redistribution of PARP upon heat shock is also concomitant with the loss of nucleosomes and the accumulation of Poly(ADP)-Ribose over the Hsp70 locus.

Using genome-wide approaches, we are assessing the generality of transcriptional regulation at the level of promoter-proximal pausing in Drosophila and mammals. Our newly developed global run-on (GRO-seq) method is an effective technology for indentifying rate limiting steps in transcription in vivo, as it maps the location, density, and orientation of transcriptionally-engaged RNA polymerases genome-wide with extremely high sensitivity. Notably, the data are revealing that the general mechanism of gene activation often involves increasing the rate of RNA Pol II escape from its promoter paused state. Our findings support that this escape of promoter-proximal paused Pol II into productive elongation is a frequently regulated step in the transcription cycle.

STEPS TOWARDS UNDERSTANDING NUCLEAR STRUCTURE AND THE PHENOMENA OF EPIGENETICS AT A MOLECULAR LEVEL

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Epigenetics encompasses changes in gene expression profiles that occur without alterations to the genomic DNA sequence of a cell. This arises from the dynamic processes that structure regions of chromosomal DNA through a range of compactions in higher eukaryotes. The structural and dynamic organization of the mammalian cell nucleus impacts many if not all aspects of chromatin regulation and gene expression. The altered pattern of gene expression is pivotal to cellular differentiation and development and is inherited by daughter cells thereby maintaining the integrity, specifications, and functions for a given cell type. Aberrancies in this epigenetic process give rise to perturbations that are also inherited and disruptive to normal cellular properties. Histone polypeptides are decorated with a large number of posttranslational modifications. It remains unclear however which of these modifications, if any, can be propagated from mother to daughter cells and what is the function(s) of these modifications in nuclear organization. We have found that modifications that function to compact chromatin, methylation of lysines 9 and 27 of histone H3 and lysine 20 of histone H4 can be inherited. The mechanism of inheritance of these modifications will be discussed as well as their function in chromosome organization.

HISTONE COVALENT MODIFICATIONS IN EPIGENETIC REGULATION

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Genomic structure and function is regulated in part through covalent post-translational modifications (PTMs) of factors and histones. The enormous variety of PTMs includes acetylation (ac), methylation (me), phosphorylation (ph), ubiquitylation (ub), and sumoylation (su). To make sense of this bewildering complexity, we focus on combinatorial patterns, temporal sequences, and cross-talk between PTMs in various experimental models, including in the yeast *S. cerevisiae* and in mammalian cells. Thus, we are trying to identify the patterns and unravel the mechanisms of these PTMs in physiological and developmental pathways, such as during gametogenesis, during replicative cell aging, and as signaling responses to cellular stress. We are also investigating potential epigenetic regulation of dramatic behavior and lifespan switches in two eusocial ant species.

BEYOND THE DOUBLE HELIX: VARYING THE “HISTONE CODE”

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Genetic and biochemical evidence have recently converged to connect epigenetic mechanisms at the level of chromatin. In addition to nucleosome remodeling and covalent modifications, eukaryotic cells generate variation in chromatin by the introduction of variant histone proteins variants, H3.1, H3.2, and H3.3, and the selective incorporation of H3 variants has been implicated in the epigenetic memory of cellular state. Using genome editing with zinc finger nucleases to tag endogenous H3.3, we have investigated the genome-wide profiles of H3 variants in mammalian embryonic stem (ES) cells and neuronal precursor cells. Genome-wide patterns of H3.3 are dependent on amino acid sequence, and change with cellular differentiation at developmentally regulated loci. The H3.3 chaperone Hira is required for H3.3 enrichment at active genes. Strikingly, Hira is not essential for localization of H3.3 at telomeres and many transcription factor-binding sites. Our studies show that Atrx and Daxx associate with H3.3 in a Hira-independent manner and that Atrx is required for Hira-independent localization of H3.3 at telomeres and for the repression of telomeric RNA. Taken together our findings demonstrate that multiple and distinct factors are responsible for H3.3 localization at specific genomic locations in mammalian cells.

HISTONE VARIANT DYNAMICS AND EPIGENETICS

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The bulk of histones are synthesized during S-phase to rapidly package newly synthesized DNA into nucleosomes, but particular variant histones are expressed at other times during the cell cycle and are deposited by distinct chaperone complexes. The replacement variant, H3.3, becomes enriched at actively transcribed genes and regulatory elements and has been implicated in transcriptional memory. H2A.Z acts as an anti-silencer in yeast, is mutually antagonistic with DNA methylation in Arabidopsis, and is enriched around promoters in diverse eukaryotes. CenH3 forms centromere-specific nucleosomes that organize and maintain the kinetochore at a unique position on the chromosome. We have biochemically characterized and directly visualized CenH3 nucleosomes and have found that they are very different from bulk nucleosomes, both in histone core structure and in DNA topology. The novel features of centromeric nucleosomes have implications for the epigenetic inheritance of centromeres and for the origin of nucleosomes from a proto-eukaryotic ancestor.

NUCLEOSOME OCCUPANCY AND TRANSCRIPTIONAL REGULATION.

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The spatial organization of chromatin and the protein composition of chromatin are both known to change during regulatory events, frequently in a causal manner. We have used a microarray-based technology to map structure across large regions of the genome. In HOX clusters, nucleosome occupancy analysis was used to identify a nucleosome-free region with characteristics similar to the sequences that target Polycomb-Group (PcG) repression in Drosophila. We find that this region is a PcG responsive repressive element in differentiating human ES cells. It contains conserved sequences and YY1 binding sites, the latter of which are required for full function. We have identified other possible Polycomb recruitment sites in human HOX using this technology. To understand the role for primary DNA sequence in determining nucleosome occupancy, we used a machine trained algorithm to generate sequence based prediction of occupancy. Comparison of the trained model with data indicates that sequence-based positioning of nucleosomes plays a greater than expected regulatory role. Structural chromatin changes in response to bacterial infection of macrophages show that the structure of regulated genes tends to mimic the predicted state following infection to a greater extent than it did prior to infection. This suggests that the energetically more favorable setting for nucleosome occupancy is the activated state. We suggest that these particular regulated genes are ‘spring-loaded’ for activation. We are examining how primary sequence might contribute to nucleosome occupancy in the HOX region. These studies demonstrate different ways that nucleosome position and occupancy can contribute to mammalian gene regulation and to the identification of novel regulatory elements.

CHROMATIN BOUNDARIES, INSULATORS AND LONG RANGE INTERACTIONS IN THE NUCLEUS

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Insulators can serve to block inappropriate interactions between both adjacent and widely separated regions of the genome. Many studies have addressed the question of how insulator activity prevents the propagation of transcriptionally silent heterochromatic structures into adjacent regions of transcriptionally active chromatin. Such insulators appear to establish a barrier that prevents the propagation of 'silencing' marks – both histone modifications and DNA methylation - past the insulator. We have identified two quite different mechanisms that contribute to barrier insulation. In order to explore how barrier insulation is established and maintained, we have studied in detail the properties of a vertebrate constitutive heterochromatic region and an associated insulator element, and find that the maintenance of the heterochromatic structure involves a dicer-dependent mechanism. In a related study, we have focused on the role of a protein factor bound to the insulator site that inhibits DNA methylation of an adjacent gene.

Another kind of insulator activity arises from the ability of certain regulatory factors bound at DNA sites distant from one another to interact and establish discrete loop domain structures. The protein CTCF plays a major role in establishing loop domains. Some of these interactions have important regulatory consequences not only for insulation but for the regulation of gene expression by control of contacts between genes and distant regulatory elements.

FLEXIBLE AND PRECISE HIGH-RESOLUTION FLUORESCENCE IMAGING OF CHROMOSOMAL REGIONS USING COMPLEX OLIGONUCLEOTIDE LIBRARIES

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Fluorescence *in situ* Hybridization (FISH) is a powerful method for studying chromosome structure, organization, and stability. However, to date, the utility of FISH has largely depended upon access to cloned template DNA used to generate probes. Clones for specific regions are often unavailable, or the genomic region of interest may contain repetitive and other non-informative sequences which can confound FISH analysis. We have leveraged our ability to chemically synthesize oligonucleotides in massively parallel reactions to produce DNA libraries that can be used to generate FISH probes. The sequences of the oligonucleotides in these libraries are selected *in silico* using empirically determined criteria so as to avoid any repetitive elements or regions homologous to other non-targeted loci, and thus targeting only the most informative elements in the genomic region to be analyzed. Our FISH protocol is similar to those used for FISH with BAC probes. We will show that using our FISH technique, human genomic regions as small as 1.8 kb and as large as whole chromosomes are successfully visualized in both metaphase and interphase cells, and we can readily obtain specific and robust signals for chromosomal regions rich in repeats and/or GC content. In addition, we will show that our oligo-FISH method can be performed jointly with immunocytochemistry of nuclear and cytoplasmic proteins, yielding a valuable tool to study nuclear and cellular architecture.

MALAT-1 NONCODING RNA REGULATES CELL MOBILITY BY MODULATING GENE EXPRESSIONS

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MALAT-1, originally identified as a metastasis associated long noncoding transcript in lung adenocarcinoma, is localized to nuclear speckles. However, functional role of MALAT-1 in metastatic characters in cancer cells has not been elucidated. Our current research has revealed that suppression of MALAT-1 expression in lung adenocarcinoma cell line by RNA interference significantly inhibited cellular mobility both in wound healing assay and in migration assay using Boyden's chamber. Moreover, we have identified metastasis/mobility-associated genes among down-regulated genes in the MALAT-1 knockdown cells by DNA microarray analysis. Quantitative analysis of pre-mRNAs has shown that the pre-mRNAs of some genes were suppressed in MALAT-1 knockdown cells but the others were not suppressed even though their matured mRNAs were found to be down-regulated. Our results suggest that MALAT-1 facilitates cell mobility by modulating the expression of metastasis/mobility-associated genes at the transcriptional and post-transcriptional stages.

TRANSCRIPTOMIC, NUCLEOCYTOPLASMIC, AND INTERACTODYNAMIC ANALYSES OF THE NUCLEAR RIBONUCLEASES RRP6 AND DIS3

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Eukaryotic cells use numerous pathways to regulate RNA production, localization, and stability, many of which are directly affected by the RNA processing exosome complex. The nuclear form of the exosome is defined by two multifunctional ribonucleases, Rrp6 and Dis3. Although we understand the general roles of these two proteins in particular RNA metabolic pathways, how they assemble into functional complexes, localize properly in cells, and regulate these pathways is just beginning to be understood. To address this matter, we have performed transcriptomic, transcript-directed, cell biological, and biochemical analyses of these *Drosophila melanogaster* polypeptides. Depletion of dRrp6 and dDis3 by RNA interference reveals that both are required for S2 tissue culture cell proliferation. In our transcriptomic analysis, we find that dRrp6 and dDis3 depletion affect distinct classes of transcripts that are found within similar pathways, with Rrp6 depletion specifically stabilizing nonsense-mediated decay substrates. In contrast, depletion of these proteins has only modest effects on the stability of *hsp70* mRNA following recovery from heat shock. We find that dDis3 interaction with dRrp6 and importin-α3 is independent of interactions with the exosome core and occurs though two independent N- and C-terminal regions. Additional dDis3 N- and C-terminal sequences contribute to subcellular distribution through putative nuclear export and retention sequences. Finally, we find that the dDis3 N-terminus is sufficient for endonuclease activity in vitro and that proper N-terminal domain structure is critical for activity of the full-length polypeptide. Our data suggest the existence of a tight and complex nuclear regulatory network involving Rrp6 and Dis3 nucleocytoplasmic transport, protein-protein interactions, and enzymatic activities that is vital for proper RNA metabolism.

SPATIAL ORGANIZATION OF THE P2 ODORANT RECEPTOR ALLELES WITHIN THE NUCLEUS OF OLFACTORY NEURONS.

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Mammals discriminate important environmental cues by means of odorant receptors expressed by olfactory sensory neurons located in the nose. Each olfactory sensory neuron transcribes only one allele from one gene chosen from a large gene family of odorant receptors. The mechanisms involved in this monoallelic expression remain elusive, but there are evidences that a cascade of transcriptional control processes are involved. We have asked whether the position of the two alleles of a given odorant receptor gene within the nucleus correlates with their transcriptional activity in olfactory neurons expressing this receptor. We are performing immuno-FISH in order to analyse the position of the P2 odorant receptor alleles in relation to transcription factories, euchromatin, heterochromatin and chromosome territories. In order to do these experiments we are using transgenic mice in which olfactory sensory neurons that express the P2 receptor also co-express the tauGFP fusion protein by virtue of IRES-mediated co-translation. In this way we can easyly identify the P2 receptor expressing neurons by the intrinsic fluorescence of GFP, or by immunofluorescence with anti-GFP antibodies. These results will elucidate whether nuclear positioning plays a role in odorant receptor gene expression. Supported by FAPESP.

BEAF 32 -CP 190 : A NEW CLASS OF INSULATORS
ROLE IN CHROMATIN ORGANIZATION AND GENE EXPRESSION

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Chromatin insulators are the DNA –Protein complexes involved in higher order chromatin organization. These are essential for the legitimate enhancer promoter interactions to regulate gene expression in cell type specific manner. The genome wide localization of these proteins using Chip-CHIP has shown Suppressor of Hairy wing(Su(HW)) , CTCF are found at specific loci while CP190 is believed recruited to same sites by protein – protein interaction. Interestingly boundary element-associated factor (BEAF-32), protein earlier identified as *SCS`* binding protein near heat shock puff at 87A also shows high association with CP190 at many chromosomal sites. We hypothesize that BEAF 32 and CP190 could constitute a another subclass of Insulators .The distribution of BEAF32/ CP190 are significantly associated with the 5' end of the genes would suggests key role in regulation of gene expression. Here we have investigated the role of BEAF 32/CP190 in organizing the genome which could interact with other classes of insulators to bring higher order structural organization in the nucleus. Genetic experiments suggest that BEAF 32 is not required for *gypsy* insulator function while CP190 mutations show compromised SCS` insulator function. The CP190 and BEAF32 show overlap in their staining patterns in an interphase nucleus and co- immunoprecipitated in cell culture. These results suggest that CP190 and BEAF 32 interactions are required for organizing a subset of the genome .

CO-TRANSCRIPTIONAL SPLICEOSOME ASSEMBLY ON MAMMALIAN MODEL GENES

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Pre-mRNA processing begins during RNA synthesis by RNA Polymerase II (Pol II), making these processes “co-transcriptional”. The degree to which 5’ capping, splicing, cleavage and polyadenylation might be functionally coupled to the transcription machinery and to chromatin is an outstanding question in the field. Prior studies in yeast showed that the splicing machinery assembles in a step-wise fashion along the length of genes *in vivo*, i.e. during the timeframe of transcription. We aim to investigate the co-transcriptional splicing process in mammalian cells, because mechanisms of alternative splicing in higher organisms are not well understood *in vivo*. This is experimentally complicated due to the complex architecture of mammalian genes. In general, numerous small exons are interspersed between ten times longer introns, making it difficult to assess how the spliceosome recognizes and assembles on an individual intron. To overcome this limitation, bacterial artificial chromosomes (BACs) harboring mouse FOS have been recombineered to produce a series of “model genes” differing in their gene architecture. Using chromatin immunoprecipitation (ChIP) of splicing factors to analyze the gene position where recruitment occurs, we show a correlation between intron position and splicing factor recruitment on single-intron-containing model genes. These data indicate that splicing signals in nascent RNA participate in the timing and location of co-transcriptional splicing factor recruitment. Surprisingly, splicing factors were also detected on an intronless FOS model gene, suggesting that either the transcriptional machinery and/or chromatin plays a role in splicing factor recruitment independent of the presence of splice sites in the nascent RNA. Our working model is that “pre-recruitment” of splicing factors facilitates early contacts with nascent RNA; subsequently, splicing factor interactions with nascent RNA are stabilized by binding to splicing signals in introns. These observations support a direct coupling mechanism between pre-mRNA splicing and active chromatin.

DYNAMICS OF HISTONE DEMETHYLASE JMJD2B

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The specific histone signature is a major determinant of transcriptionally active and silent chromatin. In particular, DNA methylation and histone post-translational modifications are central epigenetic events that have a fundamental role in chromatin biology. The balance of individual histone epigenetic marks is regulated by specific enzymes responsible for acetylation/deacetylation (HATs/HDACs) and histone methylation (HMTs/histone demethylases). Due to the important biological significance of histone modifying enzymes, we have analyzed the dynamics of JMJD2b demethylase in cells with deficiency of SUV39h HMTs and after histone hyperacetylation induced by HDAC inhibitor. Jmjd2b antagonizes H3K9me3 at pericentric heterochromatin. Thus, the recovery kinetics, as an indicator of protein-binding ability to chromatin, was studied using the FRAP technique in cells with a reduced level of centromeric H3K9 di- and tri-methylation caused by SUV39h deficiency. SUV39h wild type cells were characterized by fast recovery of Jmjd2b, which reached 80% of the pre-bleached intensity after 0.35-0.69s. SUV39h deficiency and hyper-acetylation had no effect on recovery time of Jmjd2b. We observed similar kinetic properties in cells derived from colon and prostate tumours, as in the mouse SUV39h-related experimental system. However, cells undergoing apoptosis were characterized by the specific accumulation of Jmjd2b, and no recovery after photo-bleaching was found. When we compared the dynamics of Jmjd2b with other proteins that regulate the histone signature, including HP1 and Polycomb (PcG)-related BMI-1, we observed distinct kinetic properties that were independent of protein molecular weight.

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CHROMOSOME REPOSITIONING CORRELATES WITH CHANGES IN GENE EXPRESSION DIRECTED BY TISSUE-SPECIFIC NUCLEAR ENVELOPE PROTEINS IN ADIPOCYTE DIFFERENTIATION

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The nuclear envelope has been implicated in a variety of diseases with different tissue-focused pathologies ranging from muscular dystrophy to lipodystrophy, yet the proteins mutated are widely expressed. We determined the proteomes of nuclear envelopes from different tissues to test if some of the tissue-specificity of pathology might reflect tissue differences in composition. We found that three of the novel nuclear envelope proteins we identified were upregulated in adipocytes. Overexpression of these proteins in 3T3-L1 pre-adipocytes induced expression of several adipocyte differentiation markers. In a separate screen in the laboratory, one of these, NET29, was found to recruit particular chromosomes to the nuclear periphery. We noted that several genes upregulated by NET29 overexpression in the 3T3-L1 pre-adipocytes are on chromosomes 6 and 16; so we tested for changes in positioning of these chromosomes as a result of NET29 overexpression and during pharmacologically-induced differentiation. Both chromosomes increased in peripheral localization during differentiation and also in non-induced cells that overexpressed NET29. We postulate that NET29 recruits specific genes to the periphery due to an affinity mechanism similar to that shown recently (Kumaran and Spector 2008, Reddy et al., 2008, Finlan et al., 2008) and that these genes are more active at the periphery because the adipocyte transcription factor SREBP-1 has been shown to bind to lamin A (Lloyd et al., 2005) and this might increase the local concentration of SREBP-1 at the nuclear periphery. We are currently testing the positions of the specific gene loci and the effects of NET29 knockdown on 3T3-L1 adipocyte differentiation.

LAMIN A, ITS DOWNSTREAM EFFECTORS, AND THEIR PROGNOSTIC VALUE IN COLORECTAL CANCER

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Background
Abnormalities in the expression, distribution and structural organization of A-type lamins are most commonly associated with a spectrum of inherited disorders collectively known as laminopathies. However, a novel role for lamin A has been discovered in the progression of a common epithelial cancer. Recent studies show that lamin A/C is a prognostic biomarker in colorectal cancer (CRC). Patients expressing lamin A/C are twice as likely to die of CRC cancer causes compared to clinicopathologically identical patients[1].
In vitro studies reveal that over-expression of lamin A in CRC cell lines causes up-regulation of the actin-bundling protein T-plastin, and down-regulation of the cell-adhesion molecule E-cadherin. Thus, lamin A is an upstream regulator of a pathway linking actin dynamics to loss of cell adhesion, leading to enhanced cell motility and increased invasiveness within a tumour. Here we examined the role of T-plastin as an epigenetic biomarker in CRC.
Method
T-plastin promoter methylation was analysed in human CRC cell lines, colorectal tissue, and noncancerous colon mucosa from the Netherlands Cohort Study on Diet and Cancer by using methylation-specific PCR and bisulfite sequencing. T-plastin mRNA and protein expression were studied using q-PCR and immunohistochemistry, respectively.
Results
The prevalence of T-plastin promoter methylation in human CRC was 51% (83/161) compared with 15/20 (75%) in non-cancerous colonic mucosa ($p<0.001$). Treatment of CRC cell lines with 5-Aza-2'-deoxycytidine, an inhibitor of DNA methyltransferase, caused demethylation of the promoter and increased expression of T-plastin. Immunohistochemical analysis of CRC tissue samples revealed T-plastin expression was increased compared with normal colonic mucosa. Furthermore, in T-plastin positive CRCs, the protein was strongly expressed at the leading edge of the tumour compared with its luminal counterpart. No correlation was observed between T-plastin promoter methylation and survival in a population in which lamin A expression shows a positive correlation with poor survival ($p<0.001$).
Conclusion / Future Work
T-plastin is a candidate prognostic biomarker in colorectal cancer whose expression is frequently activated by demethylation. Further research will aim to elucidate the molecular mechanisms and crosstalk between the signaling pathways that regulate motility and invasiveness in CRC.

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STC1: LINKING RNA INTERFERENCE TO CHROMATIN MODIFICATION

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In fission yeast, as in higher eukaryotes, centromeres are flanked by repetitive sequences that are packaged in heterochromatin. This heterochromatin is important for centromere function, and its formation is dependent on the RNA interference (RNAi) pathway. Non-coding RNAs transcribed from centromeric repeats are processed by Dicer to generate siRNAs. These siRNAs are loaded into Ago1, and guide the RITS effector complex to homologous centromeric transcripts. The activity of the RNAi pathway ultimately results in recruitment of the Clr4 histone methyltransferase to cognate chromatin, promoting methylation of histone H3 on lysine 9, and hence heterochromatin formation. However, what mediates the recruitment of Clr4 to sites of active RNAi is unknown.

To address this question we performed a genetic screen for novel factors required for centromeric heterochromatin integrity in fission yeast. We identified Stc1, a LIM domain protein that is specifically required for RNAi-directed heterochromatin formation. Our analyses show that Stc1 associates with the Clr4 complex CLRC, and, when RNAi is active, with the RNAi effector Ago1. Association of Clr4 with Ago1 is dependent on Stc1; moreover, tethering Stc1 to a euchromatic locus is sufficient to induce heterochromatin formation independently of RNAi. We conclude that Stc1 associates with RITS on centromeric transcripts and mediates the recruitment of Clr4, thereby coupling RNAi to chromatin modification.

FORWARD GENETIC SCREEN FOR REGULATORS OF THE SPATIAL CLUSTERING OF PERICENTRIC HETEROCHROMATIN

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The structural and dynamic organization of the mammalian cell nucleus impacts many if not all aspects of chromatin regulation and gene expression, yet little is known about the underlying molecular principles of nuclear organization. We are interested in determining these principles, as a way to understand at the mechanistic level the so far mostly correlative link between nuclear organization and gene regulation and its impact on complex phenomena such as accelerated and physiological aging. Toward this end, we are performing a forward genetic screen, using genome-wide siRNA coupled to high-throughput microscopy, for regulators of pericentric heterochromatin and its nuclear clustering. We have generated an NIH3T3 cell line stably expressing HP1α−GFP at near endogenous levels (NIH3T3-HP1α−GFP). HP1α−GFP is expressed homogenously across the cell population and the fluorescent signal is of sufficient intensity to be detected in high-throughput assays. Moreover, we show that HP1α−GFP is correctly targeted to pericentric heterochromatin clusters where it co-localizes with established markers for these structures. We validated our screening strategy by transiently knocking-down known regulators of pericentric heterochromatin, and observed disruption of HP1α−GFP foci. We expect that genome-wide RNAi on this cell line will unveil several previously unsuspected regulators of the formation and clustering of heterochromatin inside the nucleus. Candidate factors will be analyzed for their overall role in nuclear architecture and specific effects on disease models.

IDENTIFICATION OF CANDIDATE REGULATORS OF NUCLEAR ACTIN BY SEQUENCE ANALYSIS OF HUMAN PROTEINS

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Actin is among the most well characterized proteins in eukaryotic organisms and is required for many cytosolic processes, including cell motility, intracellular transport and the structural integrity of the cell. Recent reports have suggested that actin also participates in a range of nuclear processes in numerous cell types, though the role of this nuclear pool of actin is largely unknown. To identify potential regulators of nuclear actin, we performed a large scale computational screen of the human genome for proteins that contain both a putative actin binding domain as well as a nuclear localization signal. This screen resulted in 120 human proteins. Of the proteins which have known subcellular localizations in human from published immunofluoresence data, 75% have clear nuclear populations, suggesting they are true candidates. These candidates included both uncharacterized proteins as well as a proteins with known nuclear functions that have not been previously linked to nuclear actin, providing new insight into potential roles for actin in the nucleus.

THE CHROMATIN LANDSCAPE OF AN ACTIVE HUMAN KINETOCHORE RESEMBLES THE DOWNSTREAM REGION OF TRANSCRIBED GENES

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Kinetochores are specialized chromatin structures that assemble on the surface of the centromeric chromatin and are required for the proper segregation of sister chromatids during cell division. A specific histone H3 isoform termed CENP-A is found at all active kinetochores, and the identity of the chromosomal locus upon which kinetochores assemble is considered to be determined epigenetically. Recent data suggests that CENP-A molecules are arranged in distinct nucleosome domains that are interspersed with nucleosomes dimethylated on histone H3 lysine 4 (H3K4me2). However, little or nothing is known about if and to what extent the chromatin environment of active centromeres governs centromeric structure and function. Here, we employ a novel Human Artificial Chromosome (HAC) system to specifically deplete H3K4me2 at a single centromere in vivo. Our data show that severe depletion of H3K4me2 does not inactivate the kinetochore, as shown by assaying both kinetochore function and the assembly of kinetochore proteins. However, our studies go on to implicate centromeric H3K4me2 in facilitating non-coding transcriptional activity of centromeric alpha-satellite DNA at the HAC centromere. Moreover, repression of centromeric transcription at the HAC inversely correlates with the structural and functional maintenance of the local CENP-A chromatin domain. These studies provide, for the first time, evidence supporting the hypothesis that mammalian non-coding transcription forms a necessary component of centromere chromatin.

WHOLE GENOME TUMOR BISULFITE SEQUENCING EXPOSES LINK BETWEEN NUCLEAR ARCHITECTURE AND ABERRANT DNA METHYLATION IN CANCER

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DNA methylation at CpG dinucleotides is an important epigenetic mark in human development and disease. Cancer cells of various different types display aberrant methylation patterns, although these changes are only well-understood within short CpG islands. We have performed bisulfite sequencing on the Illumina Genome Analyzer technology to determine the complete methylomes of a matched pair of colon tumor and normal colon tissue from the same individual. High-coverage sequencing (30x) generated highly accurate methylation profiles at single-base resolution. The tumor sample showed dramatic and widespread DNA methylation changes organized into long contiguous chromosomal domains, and these domains strongly coincide with regions known to be physically associated with the nuclear lamina and late replication timing. We show that cancer-specific hypermethylation of CpG islands is strongly enriched within these domains, suggesting that nuclear organization could play an integral role in the aberrant methylation patterns in cancer.

A TOP2- AND HMO1-MEDIATED ARCHITECTURAL PATHWAY PROTECTS THE INTEGRITY OF TRANSCRIBED GENOMIC REGIONS DURING S-PHASE.

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Specialized topoisomerases solve topological constraints arising during chromosome metabolism. We have investigated budding yeast DNA topoisomerase II (Top2) function genomewide.

Specifically in S phase, Top2 binds intergenic regions close to transcribed genes without influencing their transcription. Top2-bound loci slow down forks and behave like fragile sites when Top2 is defective. These intergenic loci present characteristic chromatin signatures like low nucleosome density, HMG-protein Hmo1 association and histone variant Htz1 exclusion throughout the cell cycle. In *top2* mutants, Hmo1 is deleterious for viability and accumulates at pericentromeric regions in G2/M. Our data indicate that Hmo1 and Top2 bind in the proximity of certain transcribed genes in S phase suppressing the fragility of intergenic regions at the M to G1 transition.

We propose that an Hmo1-dependent epigenetic signature, together with Top2, mediates an architectural pathway controlling replicon dynamics when forks encounter transcription.

THE ROLE OF CONDENSIN IN CENTROMERE INHERITANCE

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Propagation of a functional centromere is crucial for kinetochore assembly and thus chromosome segregation. Errors in this process may lead to aneuploidy, which provokes loss of cell viability and may drive tumorigenesis. The centromeric locus, located at the primary constriction of chromosomes, is specified by the presence of nucleosomes containing the histone H3 variant CENP-A. These nucleosomes are interspersed with canonical H3 nucleosomes and the region is likely to adopt a distinct higher-order organization that is essential both for its function and its propagation.
We aim to understand the mechanisms that regulate the incorporation of CENP-A with special emphasis on those related to higher-order chromatin structure. For this purpose, we took advantage of the Xenopus egg cell-free system to develop an assay to measure CENP-A loading in chromosomes assembled in vitro. This assay recapitulates many aspects of the CENP-A deposition process recently described for human cells, e.g., it takes place after exit from mitosis and independently of DNA replication. Furthermore, we have collected evidence suggesting the functional conservation of HJURP on chaperoning CENP-A and promoting its deposition. Finally, we have studied the role of Condensin, a complex involved in chromosome condensation, in centromere architecture and propagation both in mitosis and interphase. Our results suggest that Condensin is important for proper folding of centromere chromatin and this, in turn, stabilizes CENP-A nucleosomes.

LOSS OF THE HISTONE VARIANT MACROH2A PROMOTES MELANOMA PROGRESSION.

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Cancer is a disease of both genetic and epigenetic changes. While increasing evidence demonstrates that tumor progression entails chromatin-mediated changes such as DNA methylation, the role of histone variants in cancer initiation and progression currently remains unclear. Here, we report that the histone variant macroH2A (mH2A) suppresses tumor progression of malignant melanoma. Loss of mH2A isoforms, histone variants generally associated with condensed chromatin and fine-tuning of developmental gene expression programs, is positively correlated with increasing malignant phenotype of melanoma cells in culture and human tissue samples of primary and metastatic melanoma. Knockdown of mH2A isoforms in low metastatic melanoma cells results in significantly increased proliferation and migration in vitro and growth and metastasis in vivo. Restored expression of mH2A isoforms rescues these malignant phenotypes in vitro. We demonstrate that the tumor promoting function of mH2A loss is mediated, at least in part, through direct transcriptional up-regulation of CDK8 via chromatin decondensation of its promoter. Over-expression of CDK8, a previously reported colorectal cancer oncogene, drives proliferation of melanoma cells. Taken together, our results demonstrate that mH2A is a critical regulator of chromatin structure that suppresses the development of malignant melanoma, a highly intractable cutaneous neoplasm.

IMPLICATIONS FOR EPIGENETIC CENTROMERE MARKING FROM THE STRUCTURE OF THE CENP-A/HISTONE H4 HETEROTETRAMER

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Centromeres are specified epigenetically, and the histone H3 variant, CENP-A, is assembled into the chromatin of all active centromeres. Divergence from H3 raises the possibility that CENP-A generates unique chromatin features to physically mark centromere location. The crystal structure of the sub-nucleosomal heterotetramer that we now have solved reveals three distinguishing properties: a CENP-A/CENP-A interface that undergoes a 9° rotation relative to the H3/H3 interface, a protruding loop L1 of the opposite charge as on H3, and strong hydrophobic contacts that rigidify the CENP-A/H4 interface. Residues involved in the CENP-A/CENP-A rotation are required for incorporation into centromeric chromatin suggesting specificity for an unconventional nucleosome shape. DNA topological analysis indicates that CENP-A-containing nucleosomes are nonetheless octameric with conventional left-handed DNA wrapping, in contrast to other recent proposals. Our results indicate rather that CENP-A marks centromere location by restructuring the nucleosome from within its folded histone core.

A DYNAMIC VIEW OF HIV-1 SPLICING

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Using HIV-1 as a model system, we generated cell lines containing integrated arrays of MS2-tagged reporter genes. In these lines, the HIV-1 transcription site can be visualized in live cells using fluorescent MS2 fusions. With this system, we have used microscopy techniques to analyze splicing and spliceosomal assembly. We have found that a minor fraction of HIV-1 pre-mRNAs are spliced co-transcriptionally. Consistently, large amounts of U1 and ASF/SF2 are detected at the HIV-1 transcription site, but only trace amount of the U2 and U4-U5-U6 snRNPs. Thus, only an E (early) type of spliceosomal complex is assembled at the HIV-1 transcription site.
We then analyzed the recruitment of U1snRNP, and found that it depends on the presence of a splice donor site in the HIV-1 pre-mRNA. By performing FRAP at the HIV-1 transcription site, we further found that most of U1 snRNP proteins exchange rapidly with the surrounding nucleoplasm. U1-70K was the slowest one, and its residency time at the HIV-1 transcription site was only few seconds. U1-70K mutated in the RRM recovered faster than wild-type proteins, indicating that the wild-type U1-70K is bound to U1 snRNA. Futhermore, with a Minx reporter that is spliced co-transcriptionally and recruits all the snRNPs, the dynamics of U1-70K protein was slower than in the HIV-1 model. These data suggest a model in which U1 rapidly comes on and off splice donor sequences, and would be stabilized when spliceosomal assembly occurs. The rapid dynamic of U1 may allow it to scan the entire transcriptome in search of splice donor sequences, and the specificity of spliceosomal assembly would be achieved by combinatorial interactions with other spliceosomal components, which would stabilize U1 binding.
Surprisingly, we also found that U1-C is much more dynamic than U1-70K. This suggests the provocative idea that U1C constantly comes on and off U1 snRNA. Given the interaction of U1-C with splice donor sequences, this rapid dynamic may contribute to its role in commitment complex formation.

BUDDING YEAST CHROMOSOME-ORGANIZING CLAMPS LOCALIZED TO THE NUCLEAR PERIPHERY BY TFIIIC AND MPS3

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The three-dimensional spatial architecture of chromosomes is integrally connected to chromatin function. Using the ‘chromosome-dot’ system, we investigated the subnuclear positioning of the eight known *S.cerevisiae* Extra TFIIIC (*ETC*) sites, which bind the TFIIIC transcription factor but do not recruit its cognate RNA polymerase (Pol III). Most *ETC* sites localize to the nuclear periphery throughout interphase. An *ETC* site retained tethering function when transferred to a new chromosomal location, showing that *ETC* site peripheral positioning is not context-specific. TFIIIC binding appears to be important for localization, since deleting the TFIIIC binding consensus ablated peripheral positioning. A locus-tethering assay showed that all six subunits of TFIIIC are involved, to varying degrees, in the mechanism driving peripheral tethering of *ETC* sites. Interestingly, anchoring of *ETC* sites to the nuclear periphery also required the *S.cerevisiae Sad1-UNC-84* domain protein Mps3, and specifically its nucleoplasmic N-terminal acidic domain.
In summary, we have identified *ETC* sites as a new class of genomic loci that mediate 3D spatial organization of *S.cerevisiae* chromosomes, in a manner analogous to the Chromosome-Organizing Clamps previously identified in *S.pombe*. *ETC* sites localize to the nuclear periphery through a pathway involving the TFIIIC complex and the nuclear envelope integral protein Mps3.

CHROMATIN STATES AND CELL FATE.

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A major challenge in biology is to understand how stem cells remain poised to execute the diverse gene expression programs that lead to cellular specification. The regulation of chromatin structure has emerged as a fundamental mechanism for regulating gene expression patterns and cell fate during mammalian development, however, this process is considerably complex and remains poorly understood. Embryonic stem (ES) cells provide an excellent system to study how chromatin structure can shape mammalian development because these cells can generate an unlimited number of equivalent descendants while maintaining the capacity to differentiate into any cell type in the organism. The replication-independent replacement of the major-type histones by histone variants is an important mechanism by which cells can rapidly alter chromatin states. We focused our studies on H2AZ as it has been implicated in gene regulation and because it plays an essential, but unknown role during development. While it has been well established that H2AZ incorporation can alter gene expression patterns from yeast to plants to human, how this variant contributes to specific biological outcomes is not known. We found that H2AZ is enriched at the promoter regions of a large set of genes in ES cells with known roles in development in a manner that is remarkably similar to Polycomb group (PcG) proteins. Consistent with this, H2AZ is ubiquitylated in ES cells in a Polycomb-dependent manner. H2AZ occupancy changes during ES cell differentiation suggesting that its re-distribution is necessary for cell fate transitions as loss of H2AZ leads to differentiation defects. These findings suggest that H2AZ, together with PcG proteins, may establish specialized chromatin states in ES cells necessary for the proper execution of developmental gene expression programs. We are currently investigating the detailed mechanisms by which H2AZ influences chromatin structure.

CDK PHOSPHORYLATION OF A NUCLEOPORIN CONTROLS GENE LOCALIZATION THROUGH THE CELL CYCLE

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Many inducible genes in yeast are targeted to the nuclear pore complex when active. We find that the peripheral localization of the *INO1* and *GAL1* genes is regulated through the cell cycle. Active *INO1* and *GAL1* localized at the nuclear periphery during G1, became nucleoplasmic during S-phase and then returned to the nuclear periphery during G2/M. Loss of peripheral targeting followed the initiation of DNA replication and required the cyclin-dependent kinase inhibitor Sic1. Furthermore, the Cdk1 kinase and two Cdk phosphorylation sites in the nucleoporin Nup1 were required for peripheral targeting of *INO1* and *GAL1*. Introduction of aspartic acid residues in place of either of these two sites in Nup1 bypassed the requirement for Cdk1 and resulted in targeting of *INO1* and *GAL1* to the nuclear periphery during S-phase. Thus, phosphorylation of a nuclear pore component by cyclin dependent kinase controls the localization of active genes to the nuclear periphery through the cell cycle.

SON IS ESSENTIAL FOR ORGANIZATION OF NUCLEAR SPECKLES

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Nuclear speckles provide important spatial organization and dynamic regulation for pre-mRNA processing factors in mammalian cells. While the nuclear speckle proteome is complex, little is known at the molecular level about how these factors are organized into nuclear speckles. We have discovered a new function for a large (2530 amino acid) nuclear speckle protein called Son in maintaining the organization of pre-mRNA processing factors in nuclear speckles. Depletion of Son in human cells by RNAi causes pre-mRNA processing factors to undergo dramatic reorganization into doughnut-shaped nuclear speckles. Rescue of nuclear speckle phenotype requires a unique region of multiple tandem repeat motifs in Son, while putative RNA-binding regions are dispensable for rescue. This demonstrates that the tandem repeats of Son are necessary for appropriate localization of pre-mRNA processing factors, and it suggests a potential role for Son as a nuclear speckle scaffold. Son depletion does not alter protein levels of other splicing factors, and it does not significantly alter global transcription or constitutive splicing. However, Son depletion can affect alternative splice site selection. Interestingly, Son depletion also causes cell cycle arrest at metaphase that may be related to dynamic changes in its localization during mitosis. Ongoing work aims to tease apart regulatory functions for Son in nuclear organization, pre-mRNA processing and cell cycle progression.

DIA2, AN F-BOX CONTAINING PROTEIN, IS INVOLVED IN TRANSCRIPTIONAL SILENCING

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Eukaryotic cells contain two main chromatin states: euchromatin and heterochromatin. Heterochromatin, the more dense form, is relatively gene poor and most genes found within or nearby these domains are silenced. Heterochromatin is important for the maintenance of genome integrity as it facilitates assembly of centromeres as well as aiding in the prevention of recombination of repetitive DNA sequences. Defects in heterochromatin formation and maintenance contribute towards aging and the loss of genome integrity, one of the hallmark characteristics of cancer cells. The budding yeast, *S. cerevisiae*, has three heterochromatin-like loci that are used to study basic mechanisms associated with heterochromatin formation and maintenance. These loci, which are all transcriptionally silent, include telomeres, mating-type loci (*HMR* and *HML*), and the ribosomal DNA locus. Proper gene silencing requires histone chaperones and the silent information regulator (Sir) proteins. Rtt106 is a histone chaperone with roles in the deposition of newly-synthesized H3-H4 onto replicating DNA and transcriptional silencing at telomeres and mating-type loci. A yeast genetic screen was performed to look for genes that function in parallel with *RTT106* in transcriptional silencing at the HMR locus. The dia2Δ mutation was found to enhance the phenotype of rtt106Δ cells in transcriptional silencing assays for the mating type loci and telomeres. Dia2 contains an F-box domain and is part of a Skp1-Cdc53/Cullin-F-box (SCF) E3 ubiquitin ligase complex that targets proteins for degradation by the 26S proteasome. Dia2 is known to have a role in DNA replication and the maintenance of genome integrity in yeast. Further analysis shows that Dia2 interacts genetically with other histone chaperones including Asf1, CAF-1, and Hir1 in transcriptional silencing. Our studies provide the first evidence that Dia2, an F-box containing protein, is involved in transcriptional silencing, possibly through ubiquitylating proteins involved in nucleosome assembly.

A GENETIC SCREEN TO IDENTIFY CONDITIONAL HETEROCHROMATIN MUTANTS

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The eukaryotic genome is organized into distinct structural and functional domains termed euchromatin and heterochromatin.
In S.pombe most heterochromatin is found near centromeres and telomeres. At centromeres, heterochromatin is required to maintain a high density of cohesin, facilitating proper sister chromatid segregation. The formation of heterochromatin at the centromeres of fission yeast is dependent on chromatin modifying activities and the RNAi machinery.
Heterochromatic regions are composed of repetitive sequences assembled into chromatin structures in which the nucleosomes are hypoacetylated and methylated on lysine 9 of histone H3 (H3MeK9). H3MeK9 creates a binding site for chromodomain proteins such as Swi6, Chp1 and Chp2.
Heterochromatin repeats are transcribed by RNA polymerase II and these transcripts are processed into siRNAs by the RNAi machinery.
Dissection of heterochromatin assembly pathways at the centromeres has been technically challenging because deletion of genes encoding any RNAi components or any heterochromatic factors results in the collapse of heterochromatic structures.
To overcome these technical difficulties, we performed a genetic screen to isolate temperature sensitive (TS) heterochromatin mutants. The identification and characterization of two of those mutants will be presented.

NOVEL LIVE CELL MICROSCOPY APPROACHES TO DISSECT CHROMATIN DYNAMICS IN 3D AT HIGH TEMPORAL RESOLUTION

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Higher-order chromatin structures are multi-dimensional and dynamically adapting to nuclear function and complexity, at various time scales. Imaging chromatin and nuclear organisation in individual cells is necessary to understand how chromatin folds and behaves.
We are using two new approaches to study chromosome dynamics in 3D at high temporal resolution in the yeast S. cerevisiae. First, we developed a rapid, quantitative live cell imaging method based on microfabricated mirrors. Our stereovision set-up allows for reconstruction of 3D trajectories of multiple fluorescent loci from several views of a single image up to 100 times faster than conventional methods that are based on the acquisition of stacks of images.
Extremely short acquisition times of the movement of pairs of loci enable quantification of the flexibility of the chromatin fiber under controlled experimental conditions. Assaying chromatin dynamics with 20 ms time intervals and an excellent signal to noise ratio provides a positional precision of 20 nm and allows determination of the spontaneous diffusion of specific sites along the chromosome.
Second, we visualize three independent chromosomal loci simultaneously. We created vectors to integrate repetitions of lambda repressor binding sites to which fluorescent fusion proteins between the lambda repressor and YFP can bind. Coupled to the well established lacOp and tetOp system we validate its use to determine the 3D positioning and dynamics of the three mating type loci on chromosome 3 of S.c prior and during mating type conversion.
Our studies provide new structural insights of chromatin organization.

BASE EXCISION REPAIR WITHIN NUCLEAR ARCHITECTURE : REDISTRIBUTION OF BASE EXCISION REPAIR PROTEINS IN RESPONSE TO INDUCED DNA DAMAGE.

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Eukaryotic cells are continuously exposed to metabolic and environmental stresses that damage DNA and threaten genome stability. Different DNA repair pathways have evolved and specialized for particular DNA lesions such as Single and Double Strand breaks, abasic sites and base modifications. The pathway responsible for the repair of modified bases is Base Excision Repair (BER) in which the modified base is recognized by a specific DNA glycosylase. After oxidative stress, one of the main base lesions formed in DNA is 8-oxoguanine (8-oxoG) that, if left un-repaired, induces mutations potentially involved in cancer and aging. OGG1 is the major DNA glycoylase responsible for the removal of 8-oxoG. The OGG1 activity generates an abasic site (AP) that is processed by the endonuclease APE1 that gives rise to a single strand break (SSB); polymerase and ligase activities are required to completely restore the DNA. A fine tuned coordination of the BER enzymes is crucial to avoid the accumulation of repair intermediates, such as AP sites or SSB, that would be very toxic for the cell. XRCC1 is a scaffold protein with no enzymatic activity but required for the coordination of the different steps of the BER. XRCC1 modulates the activity of the different enzymes and protects the intermediates of repair.
If the biochemistry of BER has been quite well established, the subnuclear distribution of BER enzymes in response to oxidative stress remains largely unexplored. The aim of our study is to understand the organization of the BER proteins in the context of nuclear architecture.
After induction of 8-oxoG the proteins OGG1, APE1 and XRCC1 are directed from a soluble fraction to the chromatin, following a kinetics that is in good correlation with the repair rate. Biochemical fractionation, as well as confocal microscopy studies reveal that KBrO3-induced BER foci are completely excluded from heterochromatin rich regions, and are preferentially located in less-condensed DNA areas.
If the recruitment of XRCC1 to the SSB repair foci has been largely documented, nothing is known concerning its recruitment to BER. We describe here the characterisation of XRCC1 separation of function variants that affect specifically the recruitment of the protein to BER or SSBR pathways. The molecular mechanisms mediating the recruitment of XRCC1 specifically to BER as well as the impact of this defective recruitment in terms of repair efficiency is now under analysis.

GLOBAL CHROMATIN STATE MAPS OF IPS CELLS DERIVED FROM DISTINCT ADULT SOMATIC TISSUES

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Induced pluripotent stem cells (iPSC) share all functional characteristics of embryonic stem cells (ESC) including the ability to differentiate into all three germ layers in vivo and give rise to adult chimeras with contribution to the germline. Recent work has demonstrated iPSCs are capable of generating "all iPSC" mice after tetraploid complementation assays. Several key molecular determinants of the pluripotent state have been characterized in iPS cells and appear highly similar to ES cells: 1) activation of endogenous pluripotency circuitry; 2) re-establishment of "open" chromatin at pluripotency gene promoters, including DNA hypomethylation, loss of repressive histone modifications, and enrichment of histone methylation H3K4me3; 3) at lineage-specific genes re-establishment of "bivalent domains" containing distinct histone methylation marks (H3K4me3 and H3K27me3). However recent work has suggested the molecular identity of ESCs and iPSCs may be unique and involve "iPSC-specific" gene expression signatures as well as differential DNA methylation. We have devised a novel transgenic system to examine underlying genetic and epigenetic differences between ESCs and iPSCs, with the ability to compare pluripotent cells that share genetic backgrounds yet are derived from distinct reprogramming methods. We developed a reprogrammable mouse strain by gene targeting in ES cells using a single polycistronic cassette carrying four reprogramming factors (termed "4F2A") integrated at the collagen type 1a (Col1a1) 3' UTR. In this study we compare the molecular identity of 4F2A transgenic ESCs to 4F2A iPSCs. We focused on reprogramming of two distinct tissues: adult liver and CD19+ proB cells. We analyze global gene expression profiles of tissue-specific iPSCs and their respective adult counterparts to determine if gene expression differences reveal commonly shared genes that can be correlated with an iPSC tissue of origin. In addition, using ChIP-Seq technology, we generate chromatin state maps of five histone marks, including H3K4me3 and H3K27me3, that have been previously reported for iPS cells derived from embryonic fibroblasts and adult B-cells, however we extend these analysis by analyzing additional histone marks associated with poised, distal regulatory sequences in adult tissues to determine whether these regions re-acquire an ESC-like-state during reprogramming from unique adult somatic cells. (*these authors contributed equally)

IMAGING SPLICEOSOME ASSEMBLY DYNAMICS IN LIVE-CELLS

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Splicing is known to be a co-transcriptional process, which is performed by the spliceosome, a very dynamic macromolecular complex. How spliceosome components are replaced and recycled during the process of intron recognition and removal, in vivo, remains however poorly understood. We have recently generated cell lines that allow real-time imaging of transcription and splicing in vivo. Here, we took advantage of this assay to study the dynamics of spliceosome assembly. The model consists of a U2OS-Tet-On cell line, stably transfected with tandem arrays of a modified human β-globin gene containing MS2 binding sites. After transcription induction by doxycyclin, nascent RNA molecules are visualized using a MS2 fluorescent tagged protein (mCherry-MS2). To study the dynamics of spliceosome assembly, cells were transfected with spliceosomal proteins tagged with a photoactivable-GFP. The site of β-globin transcription, identified as a nuclear "dot" labeled by mCherry-MS2, was photoactivated and GFP fluorescence intensity was monitored over time. The results show that distinct splicing proteins have different fluorescence decay curves. Our data is consistent with the view that some spliceosomal proteins such as SF1 and U2AF associate transiently with early assembly complexes, while Sm proteins have a much longer residency time in the spliceosome.

HAPLOID PLANTS PRODUCED THROUGH CENTROMERE-MEDIATED GENOME ELIMINATION

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In a cross between two species, elimination of a specific parental genome after fertilization can produce haploid offspring. Uniparental haploids can greatly accelerate plant breeding, because they yield instant homozygous lines from heterozygous parents. Despite its utility, the molecular basis of genome elimination is unknown. Centromeres are essential for chromosome segregation, yet their DNA sequences and the sequences of kinetochore proteins are fast evolving. One hypothesis to explain genome elimination states that centromeres from the two parent species interact unequally with the mitotic spindle, causing selective chromosome loss.

The centromere-specific histone H3 variant CENH3 (CENP-A in human) is required for kinetochore function in all eukaryotes. We have replaced endogenous CENH3 with altered variants in *Arabidopsis thaliana*. In one such line (GFP-tailswap), the hypervariable N-terminal tail domain of CENH3 was replaced with the tail of conventional H3.3 in a GFP-tagged protein. GFP-tailswap complemented plants were viable but largely infertile due to random chromosome segregation in meiosis. When GFP-tailswap or other CENH3 variants were crossed to wild type, chromosomes from the mutant parent were eliminated, producing haploid progeny. Maternal and paternal haploids can be generated through reciprocal crosses. Haploids were spontaneously converted into fertile diploids through meiotic non-reduction, providing a simple haploid breeding method for *Arabidopsis* researchers. We have also exploited centromere-mediated genome elimination to convert a natural tetraploid *Arabidopsis* into a diploid, reducing its ploidy to simplify genetic experiments. As CENH3 is universal in eukaryotes, our method may be extended to produce haploids in any plant species.

Our results show that CENH3 alterations can induce selective genome elimination in a cross. Future studies may reveal whether naturally occurring centromere differences can cause chromosome missegregation in hybrid zygotes.

INVESTIGATING THE RELATIONSHIP BETWEEN THE HIGHER-ORDER AND PRIMARY STRUCTURE OF CHROMATIN IN SAHFS

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We have previously identified a global and progressive heterochromatic structure within senescent human diploid fibroblasts – senescence associated heterochromatic foci (SAHFs) – which have since been widely used as a marker of senescence. Our current model is that SAHFs contribute to the altered gene expression profile seen in senescent cells. However, the structural detail of SAHFs is still unclear. Here we take cell-biological and genomic approaches to analyze in great detail how histone marks are reorganized during SAHF formation. We find that SAHFs are distinct multi-layer structures, in which H3K9me3 is enriched in the 'core' of SAHFs, surrounded by an H3K27me3 layer, which separates the core from the transcriptionally active H3K4/36me3 region. Consistent with our model, chromatin IP coupled with deep sequencing (ChIP-seq) for histone marks in these three layers reveals that these marks are dynamically redistributed at subsets of genic regions, including cyclin A and p16INK4A. Strikingly, however, the data also show that the global 'landscapes' of the silencing marks (H3K9me3 and H3K27me3) are largely unchanged, despite such a drastic alteration in chromatin structure. This indicates that the non-genic 'stable' H3K9me3 mark nucleates SAHFs, and that the global re-distribution or accumulation of H3K9/27me3 is not necessary for SAHF formation. Thus our combined approach of cell biology and biochemistry provides insight into the relationship between the higher-order and primary structures of silencing histone marks.

LAMIN A MUTANTS TARGET HP1 ISOFORMS FOR PROTEASOMAL DEGRADATION

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Mutations in the human lamin A gene cause a number of highly degenerative diseases, collectively termed laminopathies, which include Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, familial partial lipodystrophy and progerias. Laminopathic cells display characteristic abnormalities such as nuclear envelope defects and disorganization of heterochromatin. To investigate the mechanisms involved in nuclear envelope defects and heterochromatin alterations caused by lamin A mutations, we examined the effects of lamin A rod domain mutants G232E, Q294P and R386K on the distribution of heterochromatin protein 1 (HP1) isoforms α, β and γ. These lamin mutants assembled into large intranuclear aggregates, resulting in aberrant nuclear morphology and mislocalization of emerin. HeLa cells expressing these mutants showed depletion of HP1α and β without alteration of HP1γ levels. Changes in HP1α and β were not observed in cells expressing wild-type lamin A or mutant R482L, which assembled normally at the nuclear rim. Treatment with proteasomal inhibitors such as MG132 and lactacystin led to restoration of levels of HP1 isoforms and appropriate nuclear rim localization of emerin. Interestingly, this treatment also resulted in partial improvement of nuclear morphology and stable association of lamin mutants with the nuclear periphery, as deduced from fluorescence recovery after photobleaching experiments. We confirmed that inhibition of the proteasomal system by MG132 did not affect processing of the C-terminus of lamin A mutant constructs or electrophoretic mobility or levels of endogenous lamin A/C. In addition, the aberrant nuclear morphology of LAΔ50-expressing cells was not altered, suggesting that C-terminal farnesylation was not affected by MG132. We observed that HP1α and β showed increased turnover and higher basal levels of ubiquitination compared to HP1γ. Transcript analysis of components of the ubiquitination pathway showed that a specific F-box protein that is a component of the RING family of E3 ubiquitin ligases was induced in cells expressing lamin mutants. Our data indicate that mislocalized lamin mutants can induce degradation of specific heterochromatin proteins by activation of ubiquitin-mediated protein degradation.

CHROMATIN ASSOCIATIONS WITH PROMYELOCYTIC LEUKEMIA NUCLEAR BODIES IN THE INTERPHASE NUCLEUS

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The promyelocytic leukemia nuclear body (PML NB) is a dynamic subnuclear compartment. Many proteins are known to localize to these bodies implicating them in many cellular processes including transcription, apoptosis, and tumor suppression. Our previous observations linking PML NB integrity and chromatin topology suggest that PML NBs interact directly with the surrounding chromatin and may possibly perform a role in the regulation of genes on their periphery. To address whether specific gene loci are found at PML NBs, we used a tyramide-deposition based strategy to affinity purify DNA surrounding PML NBs in Jurkat cells. A previous fluorescence in situ hybridization (FISH) study had reported that in Jurkat cells the TP53 gene was associated with PML NBs. We therefore performed DNA dot blot analysis of the affinity purified DNA, covering a 6 Mbp region of chromosome 17 containing the TP53 locus. Though the TP53 locus was associated with PML NBs, we found other loci that were even more tightly associated. We used FISH to verify the associations implicated by the DNA dot blot. The associations of the loci on chromosome 17 with PML NBs were not observed in normal diploid human fibroblast cells (GM05757), indicating a cell type dependence for the PML NB association. We were able to induce the localization of the PML gene itself at a PML NB, implying a regulatory function for gene loci-nuclear body associations.

HETEROCHROMATIN PROTEINS REGULATE THE SPATIAL AND TEMPORAL DYNAMICS OF HR REPAIR AMONG REPEATED DNAS

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Heterochromatin comprises 20-30% of many eukaryotic genomes, including flies and humans. As a consequence of the chromatin compaction and abundance of repetitive sequences, DNA damage recognition and repair might be particularly challenging in this compartment. We use Drosophila cultured cells to characterize DSB repair in heterochromatin. This model system turns out to be ideal for cytological studies, since heterochromatin is organized in a very distinct subnuclear domain.
We observe that DSB induction by irradiation (IR) results in rapid and transient formation of γH2Av, Mdc1, TopBP1 and ATRIP foci in heterochromatin, revealing that this compartment is not refractory to DSB recognition and processing, opposite to what previously proposed. The presence of TopBP1 and ATRIP foci suggests that resection occurs at heterochromatic DSBs. RNAi experiments confirm that homologous recombination (HR), but not non-homologous end-joining, is involved in repairing heterochromatic DSBs. In time-lapse experiments, we observe that IR treatment results in rapid expansion and increased dynamics of the HP1a domain. This reorganization correlates with the relocation of ATRIP foci from the center to the periphery of the HP1a domain. Rad51 foci, instead, are mostly assembled outside the HP1a domain or at its periphery, where HP1a is locally removed. This suggests that heterochromatin might prevent the recruitment of the recombination machinery onto the resected DSBs at the beginning of the response, and the relocation of DSBs to the periphery might be a prerequisite for completing HR. Accordingly, the disassembly of heterochromatin by Su(var)3-9 or HP1 RNAi results in Rad51 foci formation in heterochromatin after IR. A similar effect results from the depletion of the Smc5/6 complex, that we identify as heterochromatin component, thus implying a specific function of this complex in controlling HR repair in heterochromatin.
Our results indicate that Drosophila heterochromatin is highly dynamic in response to damage. DSBs in heterochromatic sequences might be repaired by Rad51-dependent HR but only after remodeling of the heterochromatic compartment to re-localize the site of the DSB at its periphery and local displacement of HP1a.

NDC10P : A STRUCTURAL CORE OF THE INNER KINETOCHORE IN BUDDING YEAST

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Faithful chromosome segregation during mitosis is a crucial for cell survival. Budding yeast has a well-defined point centromere composed of CDEI, CDEII, and CDEIII. The CBF3 complex (Ctf13p/Skp1p/$(Cep3p)_2$/$(Ndc10p)_2$) involves in sequence-specific recognition of CDEIII DNA, and mutations of Ndc10p have shown severe defects in chromosome segregation and spindle checkpoint responses. We have determined the structure of *Kluyveromyces lactics* Ndc10p DNA-binding domain together with DNA at 2.8 Å resolution. The structural and biochemical data demonstrate that DNA-binding domains of Ndc10p dimer recruit two separated strands of DNA, and the structure reveals a structural homology with known DNA recombinases yet lacking both endonuclease and ligase activity. Moreover, we have shown that Ndc10p associates with all known inner kinetochore components (the CBF3 core complex, the Cse4p/H4/Scm3p nucleosome complex, Cbf1p, and Mif2p), and also provides a structural platform for other kinetochore proteins (e.g. the chromosomal passenger complex through Bir1p). Based on our structural and biochemical studies, we propose that Ndc10p not only binds with CDEIII DNA as a part of the CBF3 complex, but also recruits CDEI DNA through Cbf1p, and this event may initiate the stepwise assembly of kinetochore in budding yeast.

DE NOVO ASSEMBLY OF A PML NUCLEAR SUBCOMPARTMENT INDUCES DNA SYNTHESIS AT THE TELOMERE

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Cancer cells require a telomere maintenance mechanism to avoid cellular senescence induced by the replicative shortening of the chromosomal ends. Frequently, the reverse transcriptase telomerase is reactivated that can extend the telomere repeat sequence (in humans $(TTAGGG)_n$, typically 3 to 20 kb in length). However, 10 – 15 % of tumors and several immortalized cell lines use an alternative lengthening of telomeres (ALT) mechanism for the maintenance of their telomere repeats via DNA repair and recombination processes. The cytological hallmark of this process is the formation of ALT associated PML bodies (APBs) at which the promyelocytic leukemia (PML) protein assembles in a 50- to 100-nm-thick spherical shell around some telomeres [1]. How APBs assemble and whether they are a functional part of the ALT pathway is currently an open question. We have investigated the *de novo* formation of APBs by tethering structural proteins as well as DNA repair and recombination factors using an ALT positive U2OS cancer cell line that has three stable integrations of bacterial lac operator DNA sequence (*lac*O) repeats adjacent to the telomeres of chromosomes 6q, 11p and 12q [2]. A set of protein factors was identified that is able to initiate the formation of APBs in a stochastic self-organizing manner, while others are recruited only subsequently to this structure. We demonstrate that the artificially created APBs contain a functional set of DNA repair/recombination factors, and are able to induce the extension of the telomeres. These findings establish APBs as functional intermediates in the ALT pathway and make them potential targets for anti-cancer therapy in telomerase-negative tumor cells.

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DISSECTING THE FUNCTIONS OF THE CSR-1/22G-RNA PATHWAY IN CHROMOSOME SEGREGATION IN THE HOLOCENTRIC NEMATODE, *C. ELEGANS*

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A handful of small RNA-mediated activities that regulate local chromatin modification or affect the overall structure of chromosomes have been identified in various organisms, but our understanding of how small RNA pathways regulate chromatin in animals is in its early stages. We have described a small RNA pathway in *C. elegans* (the CSR-1 Pathway) that is required for proper chromosome segregation by influencing centromere formation. This pathway is dependent on the Argonaute, CSR-1 the RNA-dependent RNA polymerase EGO-1, the Dicer-related helicase DRH-3, the Tudor-domain protein EKL-1, and the β-nucleotidyl transferase protein CDE-1. Loss of CSR-1 Pathway activity leads to a failure of mitotic chromosomes to congress and orient kinetochores to opposing spindle poles.

In contrast to the case in *S. pombe*, in which repetitive element-directed small RNAs and RNAi-related components are involved in centromere formation, CSR-1 interacts with a set of small RNAs (22G-RNAs) that are antisense to thousands of germline-expressed protein-coding genes and are distributed along each chromosome. CSR-1 interacts with chromatin at its target gene chromosomal loci, but does not down-regulate target mRNA or protein levels. CSR-1/22G-RNA-targeted chromosomal domains possess an inverse correlation with the sites of HCP-3/CENP-A enrichment, and are adjacent to HCP-3 domains throughout the genome (Arshad Desai, personal communication), suggesting that CSR-1 domains are pericentromeric in nature.

We are now molecularly dissecting the functions of the CSR-1/22G-RNA pathway in modulating chromatin at target loci. We have found that the composition of histone modifications present in CSR-1 targeted regions is consistent with marks of transcriptionally active chromatin, again in contrast to *S. pombe*, where the small RNA pathway induces histone modifications characteristic of heterochromatin. Furthermore, we have determined that the CSR-1 pathway is conserved in other nematode species, with greater than 90% overlap in the groups of CSR-1/22G-RNA targeted genes. Our findings provide mechanistic insights into how the holocentric chromosomes of nematodes, in which continuous kinetochores must span the expressed domains of the genome, are assembled. Furthermore, our work emphasizes a growing awareness that AGO/small RNA pathways function in diverse organisms to provide genome-scale surveillance at the chromatin level.

MODULATION OF THE SOLUBLE POOL OF HISTONE H3-H4 BY THE HISTONE CHAPERONE NASP

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Identified in a screen for histone binding proteins in mouse testes, the nuclear autoantigenic sperm protein (NASP) is considered to be an H1 chaperone in mammalian cells. Homology with the X. laevis N1/N2 protein and S. pombe Sim3 protein, both H3-H4 chaperones, and the finding that it is associated with histones H3.1 and H3.3 in human cells in vivo, together implicated NASP in the control of H3-H4 dynamics in mammalian cells, in addition to its role as an H1 chaperone. The observation from our lab that the induction of replication stress in HeLa cells causes H3.1 to accumulate in a complex with Asf1 and NASP, which appears to buffer H3-H4 and prevent their toxic build-up in the nucleus, hinted at a particular role for NASP in controling histone H3-H4 flow during replication in cooperation with Asf1. However its precise role and position in the histone chaperone network have been unknown. The function of NASP appears to be crucial in mammals: knock-out experiments in mice suggest that it is required for embryonic development, while silencing or over-expression of NASP has been reported to affect HeLa cell proliferation. However, the molecular basis of these defects remains to be determined. We thus sought to dissect the role of NASP in detail, with a particular interest in its role in the control of H3-H4 dynamics. Using an RNAi-mediated approach to knock-down NASP in vivo, we found that NASP is required to maintain the pool of soluble H3.1/H3.3-H4 in human cell lines. Conversely, NASP over-expression lead to an increase in the soluble pool of H3-H4. While it did not appreciably affect cell proliferation under un-stressed conditions, we found that NASP depletion or over-expression had a dramatic affect on the cellular response to an acute over-supply of H3-H4. Further, depletion of NASP impaired the buffering of H3-H4 during an acute hydroxyurea-induced replication stress. We propose that NASP possesses a novel function to fine-tune the soluble pool of H3-H4, reminiscent of the role of N1/N2 protein for the storage of soluble H3-H4 Xenopus oocytes, which could be important for ensuring appropriate histone flow when normal histone supply or usage is perturbed or under conditions of acute increased demand for histones.

DISTINCT FUNCTIONS FOR THE TWO HUMAN ISOFORMS OF ANTI-SILENCING FUNCTION 1

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Histone chaperones are key actors to assemble, exchange or replace histones, the main protein component of chromatin. The histone H3-H4 chaperone Asf1 (anti-silencing function 1) is important for S-Phase progression in various organisms. While yeast present a single Asf1 isoform, two individual isoforms named Asf1a and Asf1b exist in mammals, whose precise specific or redundant roles remain largely unknown. Using both western blot, immunofluorescence microscopy and RT-QPCR, we investigated the regulation of human Asf1a and Asf1b in relation to the proliferative status in model cell lines. Using a genome-wide transcriptomic approach of cells depleted for Asf1a, Asf1b or Asf1(a+b), we assign a distinct transcriptomic signature for the two Asf1 isoforms. Furthermore, depletion of one or the other Asf1 isoform show specific phenotypes. We will present our most recent advances to support the view of a distinct task distribution between the two isoforms and discuss the physiological relevance of these findings for human diseases.

SUPERRESOLUTION LIGHT MICROSCOPY OF NUCLEAR NANOSTRUCTURE

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For the understanding of the implications of nuclear organization for the control of gene function, it is essential to improve methods of lightoptical resolution beyond the possibilities of conventional epifluorescence microscopy (optical resolution about 200 nm laterally, 600 nm axially). Here, we report on ‘nanoimaging’ of nuclear structures in mammalian cells using various types of superresolution approaches auch as 4Pi-microscopy, Spatially Modulated Illumination (SMI) microscopy, and Spectrally Assigned Localization Microscopy (SALM). While 4Pi microcoscopy was applied to superresolution of nuclear pore complex distribution and replication complexes (collaboration with Cristina Cardoso Lab), SMI microscopy made possible to measure the size of individual telomeric complexes with a resolution down to few tens of nanometer, allowing even in vivo analysis (collaboration with Karsten Rippe Lab). Using a recently developed SALM technique, Spectral Precision Distance/Position Determination Microscopy with Physically Modifiable Fluorophores (SPDMPhymod), nuclear pore complex (NPC) distribution was determined with a lateral optical resolution in the 50 nm range; the intranuclear spatial location of various types of single macromolecules in human cell nuclei was determined up to a density of ca. 1000 molecules/µm2, and distances between molecules of the same species were nanoscopically resolved down to 15 – 30 nm in optical sections of about 600 nm thickness; their spatial position was determined with an accuracy down to few nm. A quantitative analysis revealed characteristic spatial distribution patterns of individual histone, chromatin remodeller, and polymerase II molecules not observable by conventional epifluorescence microscopy (collaboration with T. Cremer Lab). Methods of theoretical polymer physics (collaboration with Dieter Heermann group) were applied to single histone nuclear distribution data to analyse expression-dependent parameters of chromatin nanostructure. It is expected that the combination of superresolution light microscopy of nuclear molecule distributions with biocomputing and nuclear biochemistry data will open an avenue to a mechanistic understanding of the complex interplay between molecular biology and functional nuclear structure.

THE EXON JUNCTION COMPLEX ASSEMBLES INTO DISCRETE NUCLEAR REGIONS, THE PERISPECKLES

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In eukaryotes, mRNA-binding proteins have major effects on mRNA metabolism by regulating their translation, degradation and subcellular localization. Understanding the molecular mechanisms by which these proteins affect the mRNA fate and the subsequent gene expression is essential. In this context, the multiprotein complex EJC (Exon Junction Complex) that is deposited onto mRNA can be considered as a critical effector of mRNA function. The EJC is loaded at the exon junction by the spliceosome and remains stably associated with mRNA after its cytoplasmic export. Structural studies showed that the core complex is formed by four proteins (eIF4AIII, MLN51, Magoh, Y14). The core complex serves as a binding platform for more than a dozen peripheral factors, playing a variety of functions.
Previous studies have shown that the EJC stimulates mRNAs translation, controls mRNA integrity and participates to motorized transport of specific mRNAs. If the central role of EJC on mRNA metabolism is clearly demonstrated, the mechanisms of the dynamic assembly and evolution of EJC remain elusive. In this study, we showed that the core and most of the peripheral EJC factors are colocalized in discrete regions of the nucleus located at the periphery of nuclear speckles. This region appears to be a novel nuclear domain that we call “the perispeckle”. By in vitro interaction analysis and in vivo experiment using FRET (Fluorescence Resonance Energy Transfer) approaches, we showed that the core factors interact together at this region. Moreover, at the ultrastructural level using CLEM (Correlative Light Electronic Microscopy), we observed that the perispeckle domain is associated with perichromatin fibers. Collectively, these data suggest that the EJC complex dynamically assembles and associates with spliced mRNAs at the perispeckles, suggesting a functional implication of this nuclear domain on gene expression.

LONG-RANGE GENE REGULATORY ARCHITECTURE OF HUMAN CHROMOSOME 21

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The spatial organization of the genome plays roles in its regulation, including the control of gene expression. This often involves direct long-range looping interactions between regulatory elements and their target genes, giving rise to complex spatial organization of chromosomes.
We determined the long-range regulatory architecture of human chromosome 21. First, to probe the overall spatial arrangement of chromosomes we used Hi-C, a technology we recently developed for mapping chromatin interactions in an unbiased, genome-wide fashion. Hi-C data shows that chromosome 21, as the rest of the genome, is compartmentalized in active and inactive spatial domains. Second, to explore the properties of three-dimensional chromatin interaction networks at higher resolution, we employed 5C technology. We generated a comprehensive long-range interaction map between 188 gene promoters and 1,252 loci distributed along human chromosome 21 and identified ~4000 specific long-range looping interactions. Analysis of this set of interactions provides new insights into the architecture of long-range control in the human genome. First, promoters are found to interact with a surprisingly large number of distant elements. Second, many distant elements can also loop to multiple promoters. Third, the interacting elements frequently contain DNAse I hypersensitive sites, predicted enhancer elements, and/or CTCF-bound elements. This suggests that our analysis identified bona fide regulatory elements interacting with promoters. Fourth, only a small fraction of the observed interactions are very frequent and span a relatively small genomic distance, whereas the large majority of interactions are infrequent and long-range (>2 Mb).
Combined, our Hi-C and 5C data provide a first view of the architecture and specificity of gene-element associations and of the potential role of higher order folding of chromosomes in facilitating gene regulation.

STEPWISE INCORPORATION OF EXOGENOUS H3.3 IN MAMMALIAN CELLS

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Chromatin regions with different transcriptional properties can be distinguished by the deposition of histone variants. In contrast to canonical H3.1 and H3.2 that are only deposited into newly replicated DNA, the histone 3 variant H3.3 is also incorporated into nucleosomal chromatin in a replication-independent manner, and accumulates predominantly at sites of active transcription. Unavailability of reliable anti-H3.3 antibodies has until recently precluded studies of endogenous H3.3. Many studies have been performed using stable cell lines continuously expressing epitope-tagged H3.3. In these systems, localization and enrichment of incorporated H3.3 in specific chromatin regions is studied at steady state. In contrast, use of fluorescent tags in transiently transfected cells enables following the behavior of newly synthesized exogenous H3.3 and its progressive incorporation into chromatin in primary cells. In the present study, we show by live cell imaging and quantitative immunofluorescence that expression of tagged H3.3 in human mesenchymal stem cells leads to its step-wise accumulation into chromatin through successive phases of 1) nucleolar enrichment, 2) targeting to dynamic foci that were found to contain histones chaperones previously implicated in H3.3 deposition, and 3) deposition into chromatin with biochemical properties comparable to that of canonical core histones. ChIP-on-chip analysis identified 1,649 H3.3-enriched promoters, a fraction of which is co-enriched in H3K4me3 alone or together with H3K27me3, while H3K9me3 is excluded. H3.3 target genes were predominantly enriched in mesodermal differentiation and signaling functions. Our data suggest that in multipotent cells, H3.3 is deposited into chromatin in a step-wide manner into lineage-priming genes with a potential for activation facilitated by H3K4me3 in facultative association with H3K27me3.

USING LOCAL PHOTODAMAGE TO STUDY INVOLVEMENT OF HETEROCHROMATIN PROTEIN 1 IN DNA REPAIR

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Photodamage can be inflicted by a focused laser beam in a selected small area of a cell. If the damage is sublethal, the cell activates repair mechanisms, therefore this approach has been used successfully to study repair of DNA [1,2]. We inflicted local DNA damage by exposing cells to ethidium anion and focused green light, in order to study the repair processes. We noticed that, in addition to known repair factors, heterochromatin protein 1 (HP1) was also recruited to the damaged area, indicating that HP1 is involved in DNA damage response (DDR) [1,2,3]. We now demonstrate that this experimental approach, although elegant and useful in studies of DNA repair mechanisms, can also lead to a false negative. If the concentration of a photosensitiser and the intensity of exciting light is sufficiently high (more than approx. 1 mW) the expected recruitment of HP1 to damage is abolished. Moreover, the total intensity of fluorescence of GFP-HP1 in the whole nucleus diminishes following damage, indicating that the concentration of GFP tagged HP1 protein decreases. It is yet unclear if this process is due to protein degradation or other mechanisms. We will discuss two potential reasons for a failure of the repair factor to accumulate at a damage site - impairing of the repair mechanism itself, and extensive scattering of exciting light [4], which leads to damage in a large area, beyond the directly illuminated region. This widespread damage results in no obvious recruitment of the repair factors to any specific nuclear region. The possibility of encountering a false negative in this type of experiment can lead to problems with data interpretation. For instance, while under some conditions HP1 recruitment was reported [3], other investigators observed dissociation of HP1 from damage [5]. We conclude that experimental conditions of inflicting local chromatin lesions must be carefully optimised in order to permit the recruitment of a repair factor to damage.

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STUDYING CENTROMERIC HISTONE DYNAMICS USING FRET

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Human CENTROMERIC PROTEIN A (CENP-A) and its homologs in other organisms are all histone H3 variants which uniquely enrich and define the centromere. Possessing both highly divergent sequences in the N and C terminal regions, these Centromeric Histone H3 varients all possess differential patterns of expression and incorporation relative to canonical H3.1. Additionally previous studies across multiple organisms have indicated that the nucleosomes formed by CENP-A deviate from the canonical H3.1 containing nucleosomes. These studies have rasied three potential CENP-A containing nucleosomes, an unstable octameric structure, a heterotypic tetramer and a homotypic tetramer. Utilizing Acceptor Photobleaching Forster Resonance Energy Transfer (AB-FRET) we have set out to measure the dynamic interactions of CENP-A with additional copies of CENP-A between two copies of CENP-A relative to H3.1 as a complimentary method to test these three models. Our initial observations show a distinct difference between the dynamics of CENP-A/CENP-A and H3.1/H3.1, with a FRET interaction not being detected in the CENP-A experiments. These results suggest that two copies of the tagged CENP-A do not co-interact within a single nucleosomes, consistent with a heterotypic tetramer model.

NUCLEATION OF NUCLEAR BODIES BY RNA

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The nucleus is a complex organelle containing numerous dynamic domains and bodies, whose functions have started to be defined; however, the molecular mechanisms for their formation are still poorly understood. Several major nuclear bodies (NB) form at sites of transcription implicating a role for RNA molecules in NB biogenesis. To assess the contribution of RNA in nuclear body formation we developed a novel experimental system. In this approach, a specific RNA tagged with a phage MS2 stem loop sequence was immobilized on a LacO-repeat array in chromatin of living cells via binding of the MS2-coat protein fused to the Lac Repressor. We used this model system to test whether specific functionally related coding and non-coding RNAs are sufficient to form major NBs. We found that tethering of the MS2-tagged replication-dependent histone gene H2b pre-mRNA to the LacO array resulted in the *de novo* formation of the histone locus body (HLB) with associated *de novo* Cajal body (CB). Impairment of the 3'-end of H2b pre-mRNA processing by deletion or mutation of conserved 3'-end *cis* elements of histone mRNA abolished the formation of *de novo* HLBs and CBs. In contrast, mutation of the 3'-end cleavage site which results in the accumulation of an uncleaved processing intermediate led to formation of *de novo* HLB and CB with higher efficiency suggesting that a build-up of H2b-MS2 pre-mRNA promotes HLB formation. To assess the ability of pre-mRNA transcripts in the formation of splicing speckles we immobilized the MS2-tagged β-globin minigene consisting of exon1, intron1, and exon2 on the array. Tethered spliced intron-containing β-globin pre-mRNAs form a protrusion towards an existing speckle or form *de novo* speckle which is in contrast to unspliced mutant and intron-less β-globin RNAs. To test the role of non-coding RNAs in NB formation we tethered NEAT1-MS2, architectural ncRNA present in paraspeckles, on chromatin and show that this is sufficient to trigger the formation of *de novo* paraspeckle. Moreover, immobilization of ectopically expressed repetitive non-coding satellite III-MS2 transcripts, normally transcriptionally hyperactivated in response to heat stress and present in nuclear stress bodies (nSBs), on the array leads to formation of *de novo* formed nSBs in the absence of a stress trigger. Collectively, we find that several types of coding and non-coding RNAs are sufficient to *de novo* assemble the specific nuclear bodies in which they are physiologically enriched. Thus, RNA may very likely act as an initial nucleation step in NB formation which serves to attract some of the freely diffusible key components of a NB and to provide a scaffold for recruitment and retention of additional NB building elements. As such, these results demonstrate that transcription is a driving force in NB formation and the resultant RNA transcripts have a pivotal role in the formation of NBs.

IDENTIFICATION OF METASTABLE RETROTRANSPOSONS IN THE MOUSE GENOME USING ACTIVE HISTONE MODIFICATION MARKS

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The majority of retrotransposons are subject to DNA methylation and subsequent repression. However, a few retrotransposons, such as IAP elements associated with the *Agouti* and *Axin-fused* loci, have been shown to partially escape this repression mechanism, and furthermore the levels of this partial repression are variable among the individuals with an identical genome sequence, resulting in 'metastable epialleles.' This discovery prompted us to investigate the possibility of finding more similarly regulated retrotransposon elements in the mouse genome. In our present study we analyzed the entire pool of retrotransposons to find out transcriptionally active retrotransposons and also to isolate the metastable (both age- and tissue-specific) ones from these active retrotransposons in the mouse genome. Here we used a novel computational method to isolate 223 retrotransposons utilizing the already available active histone modification data (H3K4me3 ChIP-seq). Extensive DNA methylation analysis in a litter of 1-week-old mice (using COBRA and bisulfate sequencing) of a sample of 7 of these putative active retrotransposons gave us at least 5 retrotransposons that are partially methylated. Further studies involving qRT-PCR and DNA methylation analysis in different ages and tissues isolated at least 4 retrotransposons that are also epigenetically metastable. Finally, the DNA methylation studies in germ cells and blastocysts helped us to categorize these active retrotransposons in 4 different groups based on the changes of their methylation pattern from the early developmental stage to the adult stage. In sum, these data confirm the presence of a large number of uncharacterized epigenetically metastable retrotransposons, and further suggest that these elements may be a major source to inter-individual variations in the mammalian epigenome.

X-LINKED GENE PROMOTERS HAVE A DIFFERENT NUCLEOSOME ORGANIZATION THAN AUTOSOMAL PROMOTERS IN *C. ELEGANS*

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We mapped nucleosome occupancy across the *C. elegans* genome in two developmental stages, embryos and adults lacking a germline. Nucleosomes were mapped by paired-end Illumina sequencing of isolated mononucleosomal DNA generated by micrococcal nuclease digestion of chromatin. We observe a difference in the nucleosome organization of X and autosomal gene promoters in both developmental stages. While both X and autosomal promoters exhibit a nucleosome-depleted region upstream of transcription start sites and a well-positioned +1 nucleosome, X promoters support higher nucleosome occupancy than autosomal promoters. No such difference is apparent at nucleosome-depleted region associated with the 3′ end of genes. The chromatin difference at promoters is partly encoded by DNA sequence, because GC content, which is correlated with higher nucleosome affinity in vitro, is higher in X-linked promoters. We also observe that nucleosome occupancy prediction by a computational model derived from *S. cerevisiae* correlates well with the observed occupancy in *C. elegans*. We are further defining the chromatin differences between X and autosomal promoters, and exploring the function of such differences. We will present our progress.

RING1B COMPACTS CHROMATIN STRUCTURE, AND REPRESSES GENE EXPRESSION, INDEPENDENT OF HISTONE UBIQUITINATION

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Genome-wide maps of histone modification are being mapped, however their role in mediating changes in higher-order chromatin structure are poorly understood. We show that polycomb repressive complexes are required to maintain a compact chromatin state at Hox loci in embryonic stem (ES) cells. There is specific decompaction in the absence of PRC2 or PRC1. This is due to PRC1, since decompaction occurs in Ring1B null cells that still have PRC2 mediated H3K27 methylation blanketing the Hox loci. Moreover, we show that the ability of Ring1B to restore a compact chromatin state, and to repress Hox gene expression in ES cells, is not dependent on its histone ubiquitination activity. We are currently investigating how Ring1B-mediated chromatin compaction limits transcription independent of polycomb's known role in mediating histone modifications.

INFREQUENT ASSOCIATIONS BETWEEN YEAST SUBTELOMERES ARE DETERMINED BY CHROMOSOME ARM LENGTH AND NUCLEAR CONSTRAINTS.

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The 3-dimensional (3D) organization of genes and chromosomes in nuclear space plays an important role in genome replication, repair and regulation of gene expression. The principles that govern this organization are currently being investigated. It is known for example that loci at large genomic distances along a same chromosome or on different chromosomes can come into contact or spatial proximity, but the nature and extent of intra-chromosomal and inter-chromosomal associations remain to be deciphered in detail. Light microscopy can be used to determine 3D distances and study the dynamics of these interactions in individual living cells. We have recently set up high-throughput 3D microscopy and image processing to unravel chromosome in vivo organization principles in the budding yeast *Saccharomyces cerevisiae* (1).
As a model system, we first used telomeric associations to analyze the principles organizing chromosome ends positions in vivo both in nuclear space and relative to representative subtelomeric references. We have determined that distances between subtelomeres are determined by chromosome arm size. Unexpectedly, we found that short distances, below 250nm, were rare and transient, as dynamic analyses substantiated. The findings that physic interactions are occasional shed a new light on chromosome end organization and might have important functional consequences. Furthermore we could show that also depending on chromosome arm length, sub-telomeres are positioned non-randomly at the nuclear periphery. Interestingly, when genomic sizes are below ~300kb, distance between subtelomeres and the yeast microtubule organizing center (SPB) consistently increase with chromosome size. Above this value dependency to arm size is lost, but the nuclear constraint due to the nuclear volume is stronger. Our data suggest that interactions between subtelomeres are neither deterministic nor stochastic and instead governed by physical constraints that include chromosome structure, centromere anchoring and nucleolar mass.

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5S RDNA PSEUDOGENES CAN MEDIATE NUCLEAR ORGANIZATION AND TRANSCRIPTIONAL REGULATION

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Mammalian genomes are largely made of repetitive elements. Although conventionally considered ‘junk DNA’ destined for silencing to prevent their spread, recent observations suggest that this notion may not be true universally, and that in many cases these elements have developed important regulatory roles for neighboring protein-coding genes. The majority of repetitive elements in mammalian genomes are derived from small RNA polymerase III transcripts, which often contain internal binding sites for pol III transcription factors. In yeast, the pol III transcription factor complex, TFIIIC, has been implicated in genome organization, chromatin structure, and the regulation of nearby protein coding genes. If this mechanism is conserved in larger eukaryotic genomes, repetitive elements, through their association with the pol III transcriptional machinery, may have a considerable impact on nuclear structure. To explore this possibility, we examined pseudogenes derived from 5S rDNA. These pseudogenes are frequently found in gene-rich regions, display striking conservation of their internal transcription factor binding sites, and are frequently bound by TFIIIC in mouse embryonic stem and trophoblast stem cells. Importantly, stable integrations of reporter constructs containing the 119bp 5S rDNA sequence cause *de novo* localization of the insertion site to the nucleolar periphery, and show decreased transcriptional activity of two linked reporter genes compared with control constructs. Therefore, integration of a 5S rDNA pseudogene has drastic effects on the positioning of a genomic region, and the transcriptional activity of nearby RNA polymerase II promoters. These data not only suggest an ancient mechanism of transcriptional regulation, but that repetitive elements, by mobilizing RNA pol III transcription factor binding sites, may play a significant role in nuclear organization and gene expression.

P63 REGULATES SATB1 TO CONTROL TISSUE-SPECIFIC CHROMATIN REMODELLING DURING DEVELOPMENT OF THE EPIDERMIS

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During development, multipotent progenitor cells establish tissue-specific programmes of gene expression that underlie the process of differentiation into specialized cell types. Special AT-rich binding protein 1 (Satb1) plays an important role in remodelling higher-order chromatin structure and regulates expression of a large number of genes in a cell type-specific manner. Here we show that during epidermal morphogenesis in mice, epidermal differentiation complex (EDC), located on mouse chromosome 3 and containing multiple genes activated during keratinocyte terminal differentiation, is recruited to the Satb1 nuclear network. Satb1 ablation results in an alteration of three-dimensional EDC structure and in a marked decrease in expression of the loricrin, involucrin, fillagrin, and other genes involved in the control of terminal keratinocyte differentiation and epidermal barrier formation. These alterations are accompanied by a significant decrease in the epidermal thickness and abnormal granular layer formation in Satb1-null mice compared to wild-type controls. Furthermore, ChIP and expression analyses revealed that Satb1 expression in keratinocytes is directly controlled by p63 transcription factor, which serves as a master regulator of epidermal development. Thus, Satb1-dependent higher-order chromatin remodelling of the EDC locus is an important process in the differentiation program of epidermal progenitor cells controlled by p63. The cross-talk between p63 and Satb1 is shown to be a novel fundamental mechanism for how master regulators of tissue morphogenesis control the fate of multi-potent progenitor cells, through establishing tissue-specific patterns of chromatin organization and remodelling.

BRCA1/BARD1 E3 UBIQUITIN (UB) LIGASE ACTIVITY IS REGULATED BY THE POLYADENYLATION FACTOR CSTF-50.

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The role of ubiquitination in RNA processing is unknown. The RING finger domains of BRCA1 and BARD1 possess significant E3 Ub ligase activity. This activity can be disrupted by breast cancer mutations of BRCA1, suggesting a relationship between the Ub ligase function and cancer development. We previously showed that the formation of the BRCA1/BARD1/CstF complex and the proteasomal-mediated degradation of RNA polymerase II (RNAP II) both inhibit polyadenylation after DNA damage. We also showed that the large subunit of RNAP IIO, but not RNAP IIA, is a specific target of the BRCA1/BARD1 ubiquitin ligase activity. Those studies indicate that BRCA1/BARD1 plays a role in the DNA damage response by direct inhibition of RNA processing and/or by degradation of RNAP II. As CstF-50 can bind BARD1, the CTD of RNAP II and Ub, it is possible that it functions to help in the assembly or stabilization of the ubiquitination complex. Consistent with this, the depletion of CstF in DT40 cells reduces the UV-induced ubiquitination of RNAP II.

To further understand the role of CstF-50 in this reaction, we determined the effect of increasing amounts of CstF-50 on the ubiquitination of different substrates by BRCA1/BARD1 in in vitro reactions. So far we have analyzed the autoubiquitination of BRCA1 and BARD1, ubiquitination of RNAP IIO and the monoubiquitination of H2A. Interestingly, CstF-50 increases the levels of BRCA1/BARD1 autoubiquitination as well as the ubiquitination of H2A and RNAP IIO. As mentioned above, CstF-50 interacts directly with the CTD of RNAP II and BARD1, and most importantly, now we have determined that CstF-50 also interacts with H2A. These results support the idea that CstF-50 might be an Ub-binding protein that is critical in determining substrate specificity for ubiquitination by BRCA1/BARD1. Interestingly, our preliminary data indicate that siRNA-mediated knockdown of CstF-50 in HeLa cells has an effect not only on the UV-induced ubiquitination of RNAP II but also on the stability of RNAP II. Currently, we are extending these studies to determine the effect of CstF-50 expression on the ubiquitination of other BRCA1/BARD1 substrates.

THE ROLE OF LAMIN A IN COLORECTAL CANCER CELL MOTILITY

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Background
We have previously shown that colorectal cancer (CRC) patients expressing lamin A/C are twice as likely to die of CRC related causes compared to clinicopathologically identical patients (1). Furthermore, expression of lamin A in CRC cell lines promotes increased cell motility (1). We now aim to identify further nucleo- and cytoskeletal proteins that regulate motility and invasiveness in CRC cells.
Methods
Cell fractions containing nucleo- and cytoskeletons (N/CSKs) were isolated from CRC cells containing either EGFP-laminA (SW480/lamA) or EGFP as a control (SW480/cntl). The cell fractions were used in 2D DIGE (Difference Gel Electrophoresis) analysis to quantify differences in protein abundance. Proteins of interest were identified using MALDI-TOF mass spectrometry.
Genome-wide Affymetrix microarray analysis of SW480/cntl and SW480/lamA cell lines was performed and genes with up or down regulated transcript levels were identified. Functional analysis of probesets showing greater than 2.5 fold differential expression was performed by Ingenuity Pathway Analysis (IPA), which produced interaction networks showing known literature-curated interactions.
Results
2D DIGE and MALDI-TOF analysis revealed that transglutaminase 2, β-actin, vimentin, actinin α4, eukaryotic translation initiation factor2 (subunit 1α) and eukaryotic translation elongation factor 1γ were up-regulated and nucleophosmin and HSP60 were down-regulated in SW480/lamA compared to SW480/cntl N/CSKs.
Microarray analysis showed that over 1200 genes had a higher than 2 fold change in expression with respect to lamin A expression. Q- PCR and RT- PCR were used to confirm the expression levels of genes from the microarray data. The most highly significant network (Network 1) produced in IPA analysis clustered together molecules linked to cancer, cellular movement and cellular growth and proliferation. Seven of the eight proteins identified in the proteomic study were shown to have known interactions with molecules in Network 1.
Future Work
Wounding assays will be used to detect the length of time between siRNA knockdown of lamin A and a change in cell motility. We will then investigate the order in which the genes of Network 1 change in expression during this time period using Q-PCR. Proteins that are found to regulate cell motility will be assessed for their potential uses as prognostic biomarkers or drug targets for CRC.
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HETEROCHROMATIN DOMAIN FIBRE REORGANIZATION OCCURS DURING LATE STAGES OF INDUCED REPROGRAMMING

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Reprogramming of induced pluripotent stem cells (iPSC) is a gradual epigenetic process that reactivates the pluripotent transcriptional network by erasing or establishing heterochromatin marks. Here, we characterize the physical structure of heterochromatin domains in full and partial mouse iPSCs by correlative Electron Spectroscopic Imaging (ESI). We show that in somatic cells and partial iPSCs, H3K9me3 marked constitutive heterochromatin is highly compartmentalized into chromocentres comprised of densely packed 10 nm chromatin fibres. In contrast, the 10 nm chromatin fibres in chromocentres of pluripotent ESCs and full iPSCs are highly dispersed in proportion to the levels of *Nanog*. This heterochromatin reorganization accompanies retroviral silencing during conversion of partial iPSCs by Mek/Gsk3 2i inhibitor treatment. To further define the structural and organizational changes to the heterochromatic domains of iPSCs, we have combined ESI with tomography, permitting the delineation of even tightly packed chromatin fibres, which would otherwise overlap in projection onto a single image plane. We find that constitutive heterochromatin domains are largely composed of 10 nm fibres, and their disruption serves as a biomarker with retroviral silencing for very late stages of somatic cell reprogramming.

FIBRONECTIN ORGANIZES SUBNUCLEAR STRUCTURE AND REGULATES GENE EXPRESSION

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Fibronectin (FN) is a dynamic glycoprotein which is involved in various cellular processes such as matrix assembly, wound healing and tumor progression. FN is known to regulate variety of genes as a result of its interaction with integrins on the cell surface. However, these genes were not thought to be direct transcriptional targets of FN since virtually nothing is known about its nuclear localization, direct DNA binding ability and role in gene regulation. Here, we provide the first evidence for FN as an active component of nucleus and particularly that of the nuclear matrix. FN is translocated into the nucleus in an NLS-dependent manner by active transport machinery involving β-karyopherin. Phosphorylation of FN plays an important role in its translocation into the nucleus. We demonstrate that FN directly binds DNA in a context- and sequence-specific manner. Gene expression profiling using MEFs derived from FN null mice revealed that FN regulates transcription of various genes involved in axon guidance, metabolism, cell to cell interaction, cancer, development and neuronal differentiation. Furthermore, these genes were validated to be direct targets of FN by genome-wide occupancy analysis. Additionally, we report that FN regulates the expression of Hox genes during retinoic acid induced neuronal differentiation. Finally, we show that FN represses its target genes by modulating the acetylation and methylation of H3K9 and H3K4. Thus, these results establish FN as a chromatin-associated factor involved in genome organization and regulation of gene expression.

CONSERVED NUCLEOSOME POSITIONING DEFINES REPLICATION ORIGINS IN YEAST S. CEREVISIAE

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In budding yeast, genome replication initiates at multiple loci termed origins of replication. The origin recognition complex (ORC) binds to the ARS consensus sequence (ACS) at these origins and during G1 phase coordinates the recruitment of several additional factors to load the replicative DNA helicase (Mcm2-7 complex) onto origins forming the pre-replicative complex. The yeast genome contains 6,000-40,000 potential ACS matches; however, only a subset of them (250-350) serves as ORC binding sites. Moreover, sequence recognition by ORC is necessary but not sufficient for origin activity, indicating that additional chromosomal features are required to specify ORC binding sites as origins of replication. Because replication initiation must occur in the context of chromatin, we investigated whether positioning of local nucleosomes is a determinant in the selection of origins of replication.

To this end, we used a combination of high-throughput sequencing and sequence analysis to precisely locate ORC binding sites and to assess the positioning of nucleosomes flanking origins of replication throughout the yeast genome. Our results revealed that yeast replication origins are characterized by an asymmetric nucleosome free region (NFR) surrounding the ACS with precisely positioned nucleosomes flanking both sides of the NFR. The ACS and adjacent sequences are sufficient to establish a nucleosome free region at origins. In contrast, both in vivo and in vitro experiments indicate that DNA occupation of the ACS by ORC is required for the canonical positioning of nucleosomes on both sides of the origin.

In conclusion, our results support a model for origin selection in yeast, which requires the interplay between nucleotide sequence and ORC: The DNA sequence establishes an asymmetric NFR that allows initial ORC binding, whereas ORC binding is required for the precise positioning of flanking nucleosomes. The resulting NFR is substantially larger than the DNA bound by ORC and we propose that the additional nucleosome-free DNA serves as the site for Mcm2-7 loading.

KINETICS AND NUCLEAR ARRANGEMENT OF POLYCOMB BODIES

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Polycomb group (PcG) proteins, organized into Polycomb bodies (PcG), are important regulatory components of epigenetic processes involved in the heritable transcriptional repression of target genes. Conversely, an antagonistic effect on transcription was ascribed to Trithorax group (TrxG) proteins that maintain the active state of chromatin. We used time-lapse confocal microscopy and FRAP modules for bleaching the individual BMI-1 foci involved in the PRC1 complex. Image acquisition was performed with the aid of white laser properties and under transmission light; however, argon laser (488 nm) was used for the FRAP technique. We bleached approximately 20 PcG foci in three independent experiments and average recovery time values with standard errors were plotted. In control GFP-BMI-1 positive cells, the 70% recovery was observed between 75-90 s, while HDAC inhibitor Trichostan A (TSA) prolonged the recovery time to 179-250 s. Our results show that histone hyper-acetylation caused by the HDAC inhibitor, TSA, can influence the diffusion of BMI-1 protein within PcG bodies. Furthermore, we obtained detailed information about the nuclear arrangement of PcG bodies after stimulation of GFP-U2OS cells by TSA.

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A MULTIFUNCTIONAL CIS-ACTING ELEMENT THAT SPECIFIES NUCLEAR REORGANIZATION, LOCUS CONTRACTION AND LOOPING OF IMMUNOGLOBULIN GENES DURING B CELL DEVELOPMENT

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We have previously shown that Sis, a silencer within the intervening region between the Jκ and the most proximal Vκ gene, is responsible for targeting Igκ miniloci carried on yeast artificial chromosomes to pericentromeric heterochromatin. We now have generated mice with a deletion of this element in the endogenous locus. Sis-/- mice exhibit normal levels of gene rearrangement and allelic exclusion, but exhibit markedly decreased distal and strikingly enhanced proximal Vκ gene usage for rearrangement. As a result, Sis-/- mice show a skewed Igκ repertoire, both in pre-B and splenic B cells. 3D FISH experiments reveal that pericentromeric recruitment is largely reduced in Sis-/- mice for both Igκ and IgH loci in pre-B cells, although pairing between Igκ and IgH loci persists. Furthermore, both Igκ locus looping and contraction are diminished in pre-B cells, providing a plausible mechanism for increased usage of proximal Vκ genes. ChIP experiments reveal that Sis possesses bound CTCF and Ikaros in pre-B cells, proteins known to mediate bridging between distal sequences with the looping out of the intervening DNA. To identify Sis-interacting sequences that may be involved in looping and setting up the repertoire, we have utilized a high-resolution circular chromosome conformation capture assay (4C) in combination with Solexa high-throughput sequencing to identify intra- and inter-chromosomal pairing partners with Sis as a function of early B cell development. Interestingly, our results reveal both pro-B and pre-B cell common and unique sets of long-range interactions. We conclude that Sis is a novel multifunctional cis-acting element.

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DISSECTING THE HUMAN KINETOCHORE - CHROMATIN INTERFACE

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Accurate cell division requires the interaction of chromosomes with the mitotic spindle at the centromere, the region of DNA where the macro-molecular kinetochore complex forms. To avoid mis-segregation, it is crucial that the kinetochore forms at only one region on each chromatid. As the formation of the kinetochore is restricted to the centromere, a long-standing question has been the nature of this site, and the genetic and molecular factors that define kinetochore assembly. The mammalian centromere is loosely defined by arrays of repetitive alpha-satellite DNA sequences. However, these sequences alone do not guarantee kinetochore formation, and additional epigenetic factors likely play a role in defining the region. Several proteins are known to localize constitutively to the centromere in human cells (collectively known as the 'constitutive centromere network' – CCAN), and are required for correct assembly of the outer kinetochore. To understand the processes of centromere specification and kinetochore assembly, we are using a combination of biochemical and cell biological approaches to dissect the interactions of the CCAN proteins with the chromatin and the outer kinetochore. We have reconstituted several CCAN sub-complexes in vitro, and are currently defining the interactions of these proteins within the CCAN network, and with centromeric DNA. By combining this biochemical data with parallel in vivo studies, we aim to further elucidate the structure of the kinetochore - chromatin interface, and the mechanisms behind centromere specification.

COORDINATION OF ENTRY INTO S PHASE WITH RIBOSOME BIOGENESIS

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Cells in log phase grow and divide, whereas those in stationary phase are quiescent. How is cell growth coordinated with cell division? We have explored this question in the yeast Saccharomyces cerevisiae. Ribosomal protein L32 is found in eukaryotes but not in bacteria. L32 binds to the initial 35S pre-rRNA to a sequence that is evolutionarily conserved in rRNA of all eukaryotes but is degenerate in bacteria. Two hours after depletion of L32 by shifting the medium from galactose to glucose, 35S pre-rRNA accumulates and rRNA processing is compromised. Large ribosomal subunits labeled with ribosomal protein L25-GFP accumulated in the nucleus after L32 depletion, showing a defect in ribosome export to the cytoplasm. Moreover, the turnover of mature ribosomes in the cytoplasm is greatly accelerated. How does the cytoplasm recognize that ribosome biogenesis in the nucleus is defective? In addition, cells depleted of L32 have an increased size. Most interestingly, the rate of cell division is slowed. FACS analysis revealed that L32 depleted cells accumulate in G1 phase, beginning 2 to 3 hours after the shift to glucose; the amount accumulating in G1 increased up through 6 hours. The accumulation in G1 is not due to loss in protein synthesis capacity of the cell, as this is not affected until several hours after the cell cycle arrest in L32-depleted cells. The transition from G1 to S phase requires that cells pass through START (yeast counterpart of the Restriction Point). In G1, Whi5 (yeast counterpart of Rb) binds to SBF (yeast counterpart of E2F) and represses activation of its target genes. At START, SBF becomes active and promotes transcription of its target genes which include CLN1/2 (yeast counterpart of cyclin E). To elucidate the mechanism by which L32 depletion and the inhibition of ribosome biogenesis arrests the cell cycle, we are investigating changes in the Whi5 pathway that are important for the G1 to S transition. For example, we have found by quantitative PCR that L32 depletion down-regulates the amount of Cln1 mRNA. Our data, together with other recent reports, show that entry into S phase is coupled with active ribosome biogenesis and the molecular mechanism underlying this coordination is beginning to be revealed. In yeast, the coordination is exerted through the Whi5 pathway (Bernstein et al. 2007 and this report), and in mammalian cells it is mediated by p53 (Pestov et al. 2001; Fumagalli et al. 2009). Therefore, ribosomal proteins may play extraribosomal functions (Warner and McIntosh 2009), including cell cycle control. (Supported by NSF MCB 0718714)

A MITOTIC HISTONE H3 MODIFICATION INTEGRATES CDK1 AND POLO-LIKE KINASE 1 ACTIVITIES TO SPATIALLY AND TEMPORALLY CONTROL THE ACTIVATION OF AURORA B

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Mitotic remodeling of chromosomes is accompanied by histone hyperphosphorylation, the functional relevance of which remains largely unknown. Here we demonstrate that histone H3 phosphorylated at threonine 3 (H3T3ph) directly recruits a crucial regulator of mitosis, the chromosomal passenger complex (CPC), to chromosomes, a step that is required for activation of its kinase subunit, Aurora B. Consistently, Xenopus egg extracts depleted of the H3T3 kinase Haspin are deficient in the chromosomal recruitment of the CPC, resulting in an impairment of Aurora B activity and a decrease in spindle size. Moreover, timely removal of H3T3ph from chromatin is critical for proper cell cycle progression. Persistence of H3T3ph at exit from M phase results in failure of the CPC to dissociate from chromosomes, leading to a delay in chromosome decondensation and nuclear re-formation. Furthermore, we demonstrate that the tight temporal control of this modification is achieved by restricting the activation of Haspin kinase to M phase. We identify Cdk1 and Polo-like kinase 1 (Plx1) as upstream activators of Haspin kinase. Cdk1 phosphorylates the N terminus of Haspin to create a Plx1 docking site. Upon binding to this conserved motif, Plx1 further phosphorylates Haspin to induce the activation of its kinase. Taken together, our data delineate a signaling pathway in which Cdk1 and Plx1 synergistically activate Haspin during M phase. Haspin phosphorylates H3T3, a mark that is “read” by the CPC and translated into an Aurora B-dependent reaction cascade on chromosomes during M phase.

53BP1, A TUMOUR SUPPRESSOR AND EARLY PARTICIPANT OF THE DNA DAMAGE RESPONSE, IS A NUCLEAR-MATRIX ASSOCIATED PROTEIN THAT CO-IMMUNOPRECIPITATES WITH LAMIN A/C IN HUMAN DERMAL FIBROBLASTS

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The p53-binding protein, 53BP1, is a critical component of the DNA damage response (DDR) machinery that safeguards genomic integrity from both endogenous stress and environmental insults, thus acting as part of an anti-oncogenic barrier[1]. Lamin A/C, encoded by *LMNA*, is a multifunctional nuclear protein with roles in both cancer and aging[2]. Furthermore, mutations in *LMNA*, or in the genes responsible for its post-translational processing, cause a broad spectrum of diseases, some of which manifest as failures of the DDR at the cellular level. One such disease, Hutchinson-Gilford Progeria, exhibits delayed recruitment of 53BP1 to sites of DNA damage, suggesting that a functional lamina is required for a correct DDR[3]. To this end, we carried out *in situ* nuclear matrix preparations of human dermal fibroblasts followed by immunofluorescence to reveal that 53BP1 is part of the nuclear matrix. As a positive control we used NuMA to show the integrity of the nuclear matrix preparation. We then performed immunoprecipitation with an anti-lamin A/C antibody of cells either left untreated (DMSO, vehicle) or treated with the DNA damage inducer etoposide (20 μM) for 30 minutes followed by recovery for 1 hour. Results showed that 53BP1 co-immunoprecipitated with lamin A/C whilst a non-lamin A/C binding protein, LAP2β, was not present in the immunoprecipitates. In the presence of etoposide, there appeared to be less 53BP1 in the immunoprecipitate, which more than likely reflects the fraction bound to chromatin, although this requires further investigation. Further work will seek to determine the exact interacting domains. Moreover, this preliminary work should serve to further understand the role of the nuclear lamina in the DDR.

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MOLECULAR MECHANISMS OF DNA REPAIR IN XP/CS CELLS

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Our genome is continually damaged either by endogenous processes or by exogenous agents (chemical agents, UV…). DNA repair mechanisms preserve the integrity of our genome. The versatile Nucleotide Excision Repair (NER) repairs the DNA damages induced, among others, by UV-light. The Transcription Factor IIH (TFIIH) is a key complex of the NER pathway and is also essential for RNA polymerase II transcription. TFIIH is composed of ten proteins, including two helicases: XPB and XPD which open the chromatin allowing the processing of both transcription and DNA repair. Mutations in TFIIH subunits are associated with a heterogeneous panel of phenotypes: the tumorigenic Xeroderma Pigmentosum (XP) which can be associated with the neuro-degenerative Cockayne's syndrome (XP/CS), and the premature aging disease Trichothiodystrophy (TTD). XP/CS patients are mutated in the Xpd gene (G602D). They are deficient in the repair of UV-induced DNA damages. Our study describes the molecular mechanism of DNA repair deficient processing in response to UV exposure in XP/CS cells. In fibroblasts isolated from XP/CS patients, we found a strong and persistent accumulation of the coating protein RPA at the DNA damage sites which triggers H2AX phosphorylation, H2A ubiquitination signal but no DNA breaks. We also found an ubiquitination of PCNA suggesting DNA synthesis after an incision in 5' of the lesion by the ERCC1/XPF endonuclease. Additionally, dynamic of TFIIH has been investigated after DNA damages in XP/CS and wild type murin cells using the microscopy approach fluorescence recovery after photo-bleaching (FRAP) in the recently produced fluorescent-TFIIH mouse model crossed with $TFIIH^{XPCS}$ mutants. Our analysis reveals that in XP/CS cells TFIIH is accumulating for many hours after UV-irradiation on the DNA damage sites and that 25% of $TFIIH^{XPCS}$ molecules are steadily bound to the damage even 6h after UV exposure. A model of action will be proposed.

THE ROLE OF PHOSPHATIDATE PHOSPHATASE LIPIN IN NUCLEAR ENVELOPE BREAKDOWN

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The nuclear envelope (NE) of metazoan cells is composed of a double lipid bilayer, nuclear pore complexes (NPCs) and the nuclear lamina, a meshwork of intermediate Lamin filaments underlying the inner nuclear membrane. The nuclear lamina provides structural support to maintain nuclear shape and is required for NPC spacing, chromatin organization, gene expression regulation, DNA replication and nuclear positioning. During open mitosis the NE breaks down to allow unperturbed segregation of chromatids. NE breakdown (NEBD) comprises several sequential steps. First, NPCs partially disassemble and holes in the NE generated by the loss of NPCs expand. Concomitantly the nuclear lamina disassembles and the NE membranes together with integral NE proteins become absorbed into the endoplasmic reticulum (ER). Little is known about what triggers NEBD, but it seems that a set of kinases including Cdk-1, PKCs, PLK-1 and Aurora A are activated in prophase and contribute to NEBD by phosphorylating proteins of the NE, NPCs and nuclear lamina. Particularly, disassembly of Lamin filaments during late NEBD is facilitated by a group of PKC isoenzymes, which requires diacylglycerol (DAG) for their activation. Lipins are conserved phosphatidic acid (PA) phosphatases that catalyze the dephophorylation of PA to yield diacylglycerol (DAG), a key element in lipid-mediated signaling. We identified the Caenorhabditis elegans Lipin homologue as an essential factor with roles in ER organization and NEBD. RNAi-mediated down-regulation of Lipin had no effect on timely entry into mitosis or on the early steps of NEBD, however Lipin was required for timely disassembly of the nuclear lamina during late NEBD. To better understand how Lipin might affect lamina disassembly, Lamin proteins isolated from control and Lipin depleted cells were biochemically analyzed. We identified several phosphorylated Lamin isoforms which were missing from Lipin depleted cells, and some of these isoforms seemed to be produced by DAG-activated PKCs. DAG-mediated signaling during NEBD and the possible evolutionary conservation of this mechanism will be discussed.

ACUTE PRELAMIN A ACCUMULATION LEADS TO ALTERATIONS IN NUCLEAR MORPHOLOGY AND FUNCTION

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Mutations in the LMNA gene have been implicated in various laminopathies that display a broad spectrum of symptoms, which seems dependent on where the mutations arise. Failure of the final processing step of immature lamin A, by the zmpste24 enzyme, leads to the accumulation of farnesylated prelamin A. Retention of this farnesylated c-terminus is believed to be the causative factor leading to some of these laminopathies. We investigated the effects of acute farnesylated prelamin A accumulation by assessing the morphology and organisation of the nucleus, stability of the nuclear lamina, effects on nuclear transport and gene expression changes. It was found that farnesylated prelamin A accumulated in cells after treatment for 48 hours with either a drug or zmpste24 siRNA and resulted in dysmorphic nuclei containing a complex nucleoplasmic reticulum (NR). Prelamin A, lamin B1 and calreticulin co-localise in the NR, which can form in interphase cells without an intervening mitosis. Treatment of farnesylated prelamin A accumulating cells with an inhibitor of CCTα, lead to a less dysmorphic nuclear phenotype and a reduction in NR. Further assessment of the morphology of nuclei at the electron microscope (EM) level lead to the demonstration that the NR in these cells consisted of single membrane bound cisternae similar to annulate lamellae. Furthermore, these structures contained regular fenestrations in both mouse embryonic fibroblasts and mouse pre-adipocytes. EM tomography revealed that these fenestrations had dimensions similar to nuclear pore complexes (NPC). Immunolabelling at the LM and EM level confirmed the presence of nup153 at these fenestrae. Quantitation of NPC on the nuclear surface after prelamin A accumulation revealed a reduction in NPC number, which was accompanied by a reduced rate of nuclear import measured using a GFP-tagged import substrate. Assessment using Fluorescence Loss in Photobleaching (FLIP) on the dynamics of GFP tagged lamin A revealed that Zmpste24 siRNA or drug treatment caused increased stability compared to control. The same technique also revealed increased peripheral binding stability of mature SREBP-1, which was accompanied by down regulated expression of targets of this transcription factor. Taken together these results show that a dysmorphic nuclear phenotype is not solely dependent on prelamin A accumulation, nuclear pore distribution is altered affecting import, lamina stability is increased and the downstream effects on SREBP-1a target genes results in decreased expression.

PROMOTER ARCHITECTURE AND SUSCEPTIBILITY TO RNA-INDUCED TRANSCRIPTIONAL GENE SILENCING

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RNAs have been shown to direct remodelling of chromatin architecture at promoters of genes, which can lead to decreased gene expression. Such transcriptional gene silencing (TGS) has therapeutic potential eg where loss of DNA methylation has resulted in increased expression of oncogenes. TGS has numerous advantages over other gene therapies given the potential for prolonged silencing following a single intervention strategy, providing silent-state chromatin is achieved and maintained at the targeted promoter. In this study, siRNAs targeting the host HIV-1 dependency factor *htatsf1* promoter were screened for TGS. No decrease in gene expression was observed, as determined by qRT-PCR. However, it is unresolved whether all genes are susceptible to TGS. Some promoters contain elements that are not conducive to nucleosome binding and it is unknown whether the RNA-induced transcriptional silencing complex (RITS) is able to override such elements to mediate TGS. To elucidate whether there are promoter characteristics associated with TGS susceptibility, promoters where TGS has been reported were examined. Criteria were applied to exclude genes where TGS was not induced by siRNAs or miRNAs, and where there was no evidence of either DNA or histone methylation, resulting in a test group of 16 genes. These were analysed for over- or under-representation of various promoter characteristics, including CpG island strength, core promoter elements, transcription start region length and histone modifications and variants. This study aims to elucidate whether there are promoter characteristics common to genes on which TGS has successfully been induced, thus facilitating the selection of TGS targets and indicating the capacity of this tool genome-wide.

IMAGING TRANSCRIPTION INITIATION OF ENDOGENOUS GENES IN LIVE CELLS

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With rapid advances in genome-wide expression analysis, there has been a resurgent interest in transcriptional regulation during differentiation and development of animal cells. Formation of the eukaryotic transcriptional pre-initiation complex (PIC) marks a highly regulated event that involves the assembly of >80 factors at the promoter of protein coding genes. Through genetics and biochemical analysis, most if not all of the proteins implicated in transcription initiation have been identified. However, in vitro biochemistry primarily informs us what is possible but not always what actually happens in the milieu of a living cell while genetics seldom reveals molecular mechanism. A powerful and complementary approach is to use modern microscopy techniques to follow protein movements within living cells.

Although imaging studies have analyzed the kinetics of sequence specific transcription factors and RNA Pol II in live cells, far less is known about dynamics of the core promoter recognition machinery. We have focused on studying the formation of the PIC at the endogenous naturally amplified histone gene locus (His) of Drosophila. Using live cell microscopy and Fluorescence Recovery After Photo-bleaching, we have been able to follow the movement of GFP tagged General Transcription Factors (GTFs) and RNA Pol II during the transcription of His genes at the beginning of S-phase.

We found that the TATA Binding Protein (TBP) binding to the His promoters is highly dependent on TFIIA but not TAFs or TFIIB. TBP-TFIIA forms an unexpectedly long-lived complex present throughout the cell cycle that likely allows multiple rounds of transcription initiation. Using this same strategy to track different components of the PIC, we are beginning to uncover unexpected features of transcription initiation that significantly alter the conventional models of transcriptional regulation and reveal a more complete picture of GTFs kinetics at a natural promoter in living cells.

H3 LYSINE 4 ACETYLATION REGULATES GENE EXPRESSION AND IS CONFINED TO PROMOTERS BY H3 LYSINE 4 TRI-METHYLATION

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Methylation of histone H3 lysine 4 (H3K4) is well conserved in evolution and robustly associated with active gene transcription. Here we show that in budding yeast, in addition to being methylated, H3K4 is also acetylated (H3K4ac). Genetic studies reveal that the histone acetyl transferases (HATs) *GCN5* and *RTT109* contribute to H3K4 acetylation in vivo, whilst removal of H3K4ac from euchromatin requires the histone deacetylase encoded by *HST1*. Genome-wide chromatin immunoprecipitation (ChIP) studies revealed that H3K4ac is enriched at promoters of actively transcribed genes upstream of H3K4 tri-methylation (H3K4me3), a pattern which has been conserved in human cells. We find that components of the Set1-containing complex (COMPASS) that promote H3K4me3 confine H3K4ac to gene promoters. In addition, genome-wide expression analyses reveal that a strain bearing an H3-K4R mutation down-regulates the expression of a subset of genes that normally have high levels of H3K4ac in their promoters and which are not affected in a *set1*Δ strain. Our results support a positive function for H3K4ac, a previously uncharacterized histone modification, in gene transcription. Furthermore, they suggest the existence of a novel form of cross-talk between two modifications on the same key residue of H3.

RPA-DEPENDENT RECRUITMENT OF HUMAN DNA HELICASE B TO STALLED REPLICATION FORKS

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Faithful genome duplication requires coordination between DNA replication and repair events through multi-tasking proteins. DNA helicase B (DHB), a robust 5'-3' DNA helicase of superfamily I conserved among vertebrates, is necessary for initiation of DNA replication in both mouse and human cells. In accordance with a potential role in DNA damage responses, human DHB (HDHB) silencing delays recovery from camptothecin treatment and elevates aphidicolin-induced chromosomal breaks. Exposure of tumor cells to genotoxins that arrest replication forks leads to accumulation of HDHB on chromatin, most prominently in S phase. This accumulation is not diminished by ATM or ATR silencing or by wortmannin treatment, suggesting that HDHB recruitment precedes or is independent of ATM/ATR. In contrast, RPA silencing reduces genotoxin-induced HDHB recruitment to chromatin. HDHB interacts physically with the N-terminal domain of RPA70 subunit (RPA70N), which serves as a recruitment scaffold for damage response proteins p53, ATRIP, Mre11, and Rad9. RPA70N interacts with an acidic motif in the DHB helicase domain that has sequence similarity to the RPA70N binding peptides from ATRIP, p53 and Rad9. This motif is conserved among vertebrate DHB proteins but absent in the bacterial superfamily 1 helicase RecD. Further analysis is in progress to test a working model that physical interaction of RPA70N with this motif recruits HDHB to sites of DNA damage during S phase. (NIH GM52948)

LACK OF CANONICAL HISTONE SYNTHESIS CAUSES CELL CYCLE ARREST AFTER DNA REPLICATION

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Replication-coupled nucleosome assembly involves the disruption of parental nucleosomes in front of replication forks and their re-assembly onto the nascent DNA. In addition, *de novo* assembly of nucleosomes from newly synthesized histones compensates for the increased demand of nucleosome formation during genome duplication. It has been suggested that the supply of histones, which affects the *de novo* nucleosome assembly, has a regulatory role in DNA replication and consequently also in cell cycle progression. To address this question *in vivo*, we generated a canonical histone null mutant (His^C) in *Drosophila melanogaster* in order to abolish *de novo* histone synthesis completely.
Due to maternally deposited histones, His^C mutant embryos initially develop without scorable defects, but they display a discrete cell cycle arrest phenotype during cell cycle 15. Surprisingly, His^C mutant cells are able to duplicate their DNA and become arrested at the S/G2 phase transition. In addition, His^C mutant cells show a significantly reduced replication rate. By successively reconstituting histone *de novo* supply through histone transgenes, we found that the replication rate of His^C mutants correlates with the histone gene copy numbers provided and thus, with the available histone supply. Moreover, our results suggest that the cell cycle arrest of His^C mutant embryos is independent of slow replication but rather reflects a novel chromatin assembly surveillance mechanism that involves transcriptional regulation of the CDC25C phosphatase String.
The lack of *de novo* histone synthesis of His^C mutants can be fully rescued by 12 copies of histone transgenes. This combination of the His^C mutant with a complete transgene-based rescue provides a novel genetic whole organism assay system for higher eukaryotes in which questions concerning the biological significance of canonical histones, histone variants and histone modifications can be directly assessed.

INTERACTIONS BETWEEN THE DNA DAMAGE AND SPINDLE CHECKPOINTS IN BUDDING YEAST

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A single double-strand break (DSB) is sufficient to arrest budding yeast prior to mitosis through the action of the DNA damage checkpoint. Arrest is eventually attenuated and cells adapt, after about 6 cell-division times. Deletion of *MAD2* or other spindle checkpoint genes shortens the length of arrest. We showed that Mad2 acts by sensing a DNA damage-propagated signal (apparently the histone modification γ-H2AX) from the DSB to the centromere. Deleting the centromere on the same chromosome mimics *mad2*Δ. However another way of inactivating the centromere, by inducing transcription across it, has the unexpected opposite effect: cells remain permanently arrested, whereas transcription across *CEN3* by itself does not perturb cell cycle progression. In this case the DSB can be on a chromosome different from the transcribed centromere. Again, deletion of MAD2 suppresses permanent arrest. These data suggest that transcription across a centromere creates a sub-threshold spindle checkpoint signal that is greatly amplified by activating the DNA damage checkpoint.

A number of mutations in a variety of cellular functions cause permanent checkpoint-mediated arrest; these include mutations that affect the intensity of the damage signal (affecting 5' to 3' resection of DSB ends, binding to single-stranded DNA) and others affecting turning off the checkpoint (dephosphoryation of activated checkpoint kinases, mutations in Polo kinase, etc.). Another class of adaptation-defective mutations ablate steps in retrograde vesicle transport, including *gcs1*Δ and *arf1*Δ and the mutations of the GARP complex (e.g. *vps51*Δ). These mutations alter the localization of separase (Esp1) and its securin/chaperone, Pds1; but their defects can be overcome by attaching a SV40 nuclear localization signal to Esp1, re-establishing it in the nucleus. Alterations in Pds1 and Esp1 localization can also be detected in wild type cells suffering a single DSB. These results suggest that there is a dynamic regulation of separase activity that involves its transport and localization in the nucleus.

HETEROCHROMATIN DYNAMICS: A NETWORK OF MOBILE AND STABLE PROTEINS FORMS A MAJOR REPRESSIVE CHROMATIN DOMAIN.

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Heterochromatin plays important roles for gene silencing and maintenance of genomic integrity. Repressive chromatin domains are established through distinct combinations of histone- and DNA-modifying enzymes and proteins which recognize these modifications. A hallmark of pericentric heterochromatin is a combinatorial histone methylation signature which is established in a sequential pathway. The histone methyltransferase Suv39h establishes histone H3 lysine 9 trimethylation (H3K9me3). This modification is bound by HP1 proteins which, in turn, recruit Suv4-20h methyltransferases to establish the other histone modification, H4K20me3. To better understand the dynamic interplay between Suv39h, HP1 and Suv4-20h at heterochromatin we determine their concentrations in the cell and their dynamic parameters. EGFP knock-in alleles for Suv4-20h enzymes confirm that at endogenous expression level both proteins are enriched at pericentric heterochromatin, however, their concentration is extremely low. In order to determine mobility parameters we developed a novel tool for inducible expression of tagged proteins in mouse embryonic stem cells. Our data demonstrate that, in agreement with previous reports, HP1 is a highly mobile protein. Suv39h and Suv4-20h enzymes, in contrast, are very stable components of heterochromatin. This is surprising as Suv4-20h enzymes are recruited to heterochromatin in an HP1-dependent manner. Thus, additional interactions are required for anchoring these proteins. Analyses of localization and mobility parameters of different Suv4-20h truncations in combination with immunoprecipitation assays will now reveal the domains and interaction partners which mediate this stable association with heterochromatin.

FEATURES OF SPATIAL NUCLEAR ORGANIZATION IN DYNAMIC GENE EXPRESSION

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The non-random spatial organization of genes in the interphase nucleus plays an important role in establishment and regulation of gene expression. However, little is known about genomic features of associating loci and how they relate to rapid transcriptional regulation by an inducible transcription factor. We have examined spatial organization at Glucocorticoid Receptor (GR)-responsive loci by chromosome conformation capture on chip (4C) technique. Our interaction profiling was combined with genome-wide expression, protein occupancy, and chromatin accessibility profiles, to investigate the properties of the spatial organization. We report that the nucleus is pre-organized in a conformation allowing an efficient transcriptional reprogramming by GR. The genomic regions in physical proximity with either locus were gene-rich and mostly disjoint from each other. Both GR-induced and GR-repressed genes were found to co-localize in the same hubs. Although the hubs were not enriched for GR regulated genes or any functional group of genes, they were highly enriched for GR binding sites and DNaseI hypersensitive sites. These findings suggest that clustering of regulatory elements is the major feature of such nuclear hubs. These nuclear sub-environments are poised to respond to diverse signals in general, and permit efficient gene regulation to exert diverse gene-specific expression programs.

BIASED DISTRIBUTION OF THE NUCLEAR PORE COMPLEX CONTRIBUTES TO NUCLEAR DIFFERENTIATION IN CILIATE TETRAHYMENA

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The ciliated protozoa have two functionally and structurally distinct nuclei in a single cell. A somatic macronucleus (MAC) is transcriptionally active while a germ line micronucleus (MIC) is inert during vegetative growth. Understanding such "nuclear dimorphism" is a central issue in ciliate biology, but molecular bases of nuclear differentiation remain largely unknown. To understand them, we have studied structural differences of the nuclear pore complex (NPC) between two nuclei in Tetrahymena. We identified 13 nucleoporins, including Seh1 and Nup98, based on localization of GFP fusions. Most of the nucleoporins were localized to both MAC and MIC. Interestingly, however, four species of Nup98 showed strikingly differentiated localization: two of them were localized exclusively to MAC (MacNup98A and B), and the other two exclusively to MIC (MicNup98A and B). A unique feature of MicNup98 is that they have novel NIFN repeats whereas MacNup98s have ordinary GLFG repeats conserved in Nup98 from yeasts to humans, suggesting that particular nucleoporins or the NPC might be involved in cell differentiation. Previously, we reported that MacNup98 and MicNup98 control protein trafficking to each of the nuclei (Iwamoto et al., Curr. Biol., 2009). To further understand involvement of the NPC in differentiation, we have first developed a method of fluorescence live-cell imaging to monitor dynamic behavior of specific molecules in immobilized living Tetrahymena cells undergoing nuclear differentiation. For high-resolution imaging, we also developed a method of live CLEM (correlative light and electron microscopy after live imaging) in which the same cell observed by fluorescence microscopy can be observed by electron microscopy. Using these imaging technologies, we found that the NPCs moved toward the anterior side of the nuclear envelope during second postzygotic mitosis to generate two distinguishable nuclei - one NPC-rich and another NPC-poor. Live-cell imaging further showed that these NPC-rich and NPC-poor nuclei were destined to become a new MAC and MIC, respectively. During the nuclear differentiation, MAC-specific MacNup98B was incorporated in the NPCs of the newly differentiating MAC nucleus while MIC-specific MicNup98A was lost. These results suggest that components of the NPC may contribute to nuclear differentiation through regulation of selective nuclear transport.

TFIIH KINASE IS A GENERAL REGULATOR OF RNA POLYMERASE II TRANSCRIPTION BUT REGULATES CAPPING ONLY ON SPECIFIC GENES IN MAMMALIAN CELLS

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RNA polymerase II C-terminal domain Ser5 phosphorylation by the TFIIH kinase has been considered a requirement for various steps following promoter escape including mRNA capping. However, recent work has questioned the requirement of TFIIH kinase in general transcription particularly in mammalian cells. In the work presented here, conditional deletion of the Mat1 subunit of the TFIIH kinase in mouse embryo fibroblasts results in a general reduction of both Ser5 phosphorylation and transcription rate, which unexpectedly is not reflected as decreased steady-state mRNA levels. Defective mRNA capping and destablilization of Pol II in promoter regions was only noted on a small set of genes accompanied with a severely reduced transcription rate and reduced mRNA levels. These results demonstrate that the TFIIH kinase is the major Ser5 kinase in mammalian cells regulating Pol II transcription rate globally but capping only on specific TFIIH kinase-sensitive genes. The uncoupling of Pol II transcription rate from steady-state mRNA levels suggests that regulation of transcript stability may play a more significant role in mammalian gene regulation than presumed.

NUCLEAR STRUCTURAL NETWORKS: LAMIN A/C PROVIDES HARDWARE FOR NUCLEAR LAMINA CONSTRUCTION AND REGULATION OF GENE EXPRESSION

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Lamins are intermediate filament (IF) proteins specialized to form two-dimensional networks at the inner nuclear membrane (INM) of every metazoan cell nucleus, as impressively seen in the giant cell nuclei of Xenopus laevis oocytes. The establishment of such regular and extended lamin fiber systems as well as their degree of connectivity is extensively regulated by factors within the INM, such as emerin and lamin B receptor (LBR). Thereby a structure is formed that has been called by Blobel and colleagues “the nuclear lamina”. Moreover, lamins coordinate interactions that occur specifically in somatic cells, i.e. the association of the nuclear envelope with components of interphase chromatin such as histones and the barrier-to-autointegration factor (BAF). Recently, mutations in lamin A have been demonstrated to cause a bewildering number of different human diseases such as Emery-Dreifuss muscular dystrophy (EDMD), cardiomyopathy and premature ageing. The various pathomechanisms may in part depend on structural changes of the nuclear envelope, however, they may also relate to the specific association of lamin A/C with distinct signalling molecules, transcription factors and chromatin. In addition, it has been hypothesized that a widespread A-type lamin system may functionally organize architecture within the cell nucleus. We have now established conditions to renature recombinant lamin A and generate homogenous complexes as demonstrated by analytical ultracentrifugation. Thereby, we were able to develop robust protocols to analyze the potential interactions these molecules are engaged in to form fibrillar structures. Furthermore, we generated various truncation mutants which allowed us to investigate the contribution of individual segments of lamin A/C for molecular interactions. Thereby we were also able to investigate in molecular detail at which level of organization wild-type and mutated lamin A molecules differ.

MECHANISM AND REGULATION OF DNA REPLICATION DURING THE CELL CYCLE AND IN RESPONSE TO DNA DAMAGE

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The eukaryotic cell cycle coordinates the accurate duplication and segregation of the genome during proliferation. The large genomes of eukaryotic cells are replicated from multiple replication origins during S phase. These origins are not activated synchronously at the beginning of S phase, but instead fire throughout S phase according to a pre-determined, cell type specific program. Only after the entire genome is completely replicated do cells proceed into mitosis.

Ensuring that each origin is efficiently activated once and only once during each S phase is crucial for maintaining the integrity of the genome. This is achieved by a two-step mechanism. The first step, known as licensing, involves the loading of the Mcm2-7 proteins into pre-replicative complexes (pre-RCs) at origins. We have recently reconstituted this reaction with purified proteins and I will discuss insights into the mechanism of licensing as well as the architecture of the pre-RC.

Pre-RCs, which are essential for initiation, can only assemble at origins during G1 phase when cyclin dependent kinase (CDK) activity is low because CDKs inhibit each pre-RC component individually. Deregulation of CDK activity, a common feature of cancer cells, induces genomic instability because licensing is compromised. Insights into how reductions in licensing can induce DNA damage will be presented.

CDKs trigger initiation by phosphorylating two essential proteins, Sld2 and Sld3. A second protein kinase, Cdc7, along with its regulatory subunit, Dbf4, is also required for initiation. Origin firing is inhibited by a third protein kinase, Rad53, which is activated in response to DNA damage. The mechanism by which Rad53 inhibits origin firing will be described which involves the phosphorylation of Sld3 and Dbf4.

SPACE AND TIME IN THE NUCLEUS: DEVELOPMENTAL CONTROL OF REPLICATION TIMING AND CHROMOSOME ARCHITECTURE

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All eukaryotic cells replicate segments of their genomes in a defined temporal sequence. In multicellular organisms, at least half the genome is subject to changes in this temporal sequence during development. We now know that this temporal sequence and its developmentally regulated changes are conserved across distantly related species, suggesting that it either represents or reflects something biologically important. However, both the mechanism and the significance of this program remain unknown. We recently demonstrated a remarkably strong genome-wide correlation between replication timing and chromatin interaction maps, stronger than any other chromosomal property analyzed to date, indicating that sequences localized near each other replicate at similar times. This provides molecular confirmation of longstanding cytogenetic evidence for spatial compartmentalization of early and late replicating DNA, and supports our earlier model that replication timing is re-established in each G1-phase coincident with the anchorage of chromosomal segments at specific locations within the nucleus (Timing Decision Point; TDP). We will review the evidence linking the replication program to the 3 dimensional architecture of chromatin in the nucleus, and discuss what such a link might mean for the mechanism and significance of a developmentally regulated replication program.

EPIGENETIC CHALLENGES IN CENTROMERE INHERITANCE DURING THE CELL CYCLE

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Studies concerning the mechanism of DNA replication have advanced our understanding of genetic transmission through multiple cell cycles. Recent work has shed light on possible means to ensure the stable transmission of information beyond just DNA and the concept of epigenetic inheritance has emerged. Considering chromatin-based information, key candidates have arisen as epigenetic marks including DNA and histone modifications, histone variants, non-histone chromatin proteins, nuclear RNA as well as higher-order chromatin organization. Thus, understanding the dynamics and stability of these marks following disruptive events during replication and repair and throughout the cell cycle becomes of critical importance for the maintenance of any given chromatin state. To approach these issues, we study the maintenance of heterochromatin at centromeres, key chromosomal regions for the proper chromosome segregation. We wish to define a possible framework for an understanding of both the stability and reversibility of epigenetic marks and their dynamics at centromeres. We will present our most recent work on this topic.

THE ORIGIN RECOGNITION COMPLEX (ORC) IS REQUIRED FOR CHROMOSOME SEGREGATION DURING MITOSIS

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Replication of the human genome prior to each cell division is a highly coordinated process. Accurate inheritance of genetic information requires precise coordination and execution of DNA replication and chromosome segregation during each cell division cycle. Loading of the ORC protein subunits onto replication origins is the earliest step in DNA replication, but in human cells ORC subunits do not remain as a single complex throughout the cell cycle. For example, the largest ORC subunit, Orc1 is degraded at the G1/S transition and also controls cyclin E-CDK2-dependent centrosome duplication. Orc2 and Orc3 serve as core subunits of the ORC complex. In addition to their role in DNA replication, Orc2 and Orc3 form a separate stable sub-complex and localize to centrosomes and centromeres during the entire chromosome inheritance cycle. Previous data demonstrated that Orc2 or Orc3 depletion by RNA interference caused 30% of the cells to arrest in a pseudo-mitotic state, with abnormally condensed chromosome structure, failure of stable spindle attachment of chromosomes and multiple centrosomes. Orc2 and Orc3 associate with a subset of kinetochore proteins only during mitosis, including BubR1, CenpE and CenpF and in the absence of Orc2 or Orc3, spindle attachment is comprised, even though the spindle assembly checkpoint appears to have been satisfied. Orc2 co-localizes to centromere with the inner centromere protein CenpA and Orc3 binds directly to the heterochromatin protein HP1. In the absence of Orc2, centromere associated satellite repeat DNA becomes decondensed and HP1 no longer localizes to pericentric heterochromatin. We suggest that in the absence of Orc2/3, centromere structure is compromised, causing spindles to attach to kinetochores transiently, satisfying the spindle assembly checkpoint, but stable spindle attachment is then lost and cells arrest in a pseudo-early anaphase state. Since Orc2 and Orc3 participate directly in centromere activity and chromosome segregation during mitosis beyond their known roles in the initiation of DNA replication, we suggest that there is an ancient link between origins of DNA replication and chromosome segregation.

HOW MAMMALIAN TELOMERES SOLVE THE END-PROTECTION PROBLEM

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For reasons that are unclear, eukaryotes evolved linear chromosomes, despite the problems associated with chromosome ends. Most eukaryotes use telomerase to solve the ‘end-replication problem’, which refers to the inability of the canonical DNA replication machinery to duplicate the ends of linear DNA. Our work focuses on the ‘end-protection problem’: the requirement for chromosome ends to remain protected from the DNA damage response pathways that can detect and repair broken chromosomes. In mammalian cells, double-strand breaks (DSBs) activate the ATM and ATR kinase signaling pathways and undergo repair through non-homologous end-joining (NHEJ) and homology-directed repair (HDR). Mammalian telomeres repress each of these unwarranted events through the agency of a six-subunit complex, called shelterin. Shelterin is endowed with specificity for telomeres not because it recognizes the end of the chromosome but as a consequence of the sequence preference of several DNA binding factors in the complex. Three shelterin proteins bind to the TTAGGG sequences either in double-stranded DNA (TRF1 and TRF2) or in single-stranded form (POT1). As TRF1, TRF2, and POT1 are held together by protein-protein interactions, the selectivity of shelterin for telomeric DNA is exquisite.
The logic of the mammalian telomere system is that telomerase ensures the presence of shelterin binding sites at chromosome ends, thereby enabling shelterin to guard the natural ends of chromosomes from the DNA damage response. In turn, shelterin is thought to promote and regulate telomerase in cis, ensuring that the enzyme does not add telomeric DNA to broken ends. Based on conditional knockout experiments in mouse cells, we have established how the six shelterin subunits repress the DNA damage response. The data show a great degree of compartmentalization in shelterin. The repression of the ATM kinase signaling pathway and NHEJ repair are primarily the function of TRF2. The ATR kinase signaling pathway is repressed by the POT1 proteins which are bound to the rest of shelterin through protein interactions involving TIN2 and TPP1. Inappropriate HDR at telomeres is repressed by a concerted action of Rap1 and POT1. The sixth component of shelterin, TRF1, acts to promote the semi-conservative replication of telomeric DNA. In the absence of TRF1, replication of the telomeric repeats falters and telomeres behave like the common fragile sites. Progress on the mechanism by which shelterin proteins repress the various DNA damage response pathways will be presented.

THE SMC5/6 COMPLEX – RESOLVING TOPOLOGICAL TENSION DURING CHROMOSOME REPLICATION?

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Structural Maintenance of Chromosome (SMC) protein complexes are central for genome stability and are required for both correct chromosome segregation and DNA repair. There are three different SMC complexes in eukaryotic cells; cohesin, condensin and the Smc5/6 complex. Cohesin and condensin have relatively well established roles in sister chromatid cohesion and chromosome condensation, respectively. The function of the Smc5/6 complex remains, however, unclear. We have determined the chromosomal association pattern of the budding yeast Smc5/6 complex during different cell cycle phases, and after induction of DNA damage. The complex interacts with centromeres and intergenic regions along chromosome arms in the duplicated genome, and is recruited to DNA double-strand breaks induced during replication or in G2. Interestingly, the frequency of arm-interaction sites is determined by chromosome length and structure. Moreover, mutations of the complex lead to a chromosome-length specific replication defect. A similar phenotype was detected in cells lacking type I topoisomerases, and in vivo plasmid-based assays provide evidence for a role of the Smc5/6 complex in the resolution if replication-induced topological stress. This investigation, and the implications it has on the view of how the Smc5/6 complex maintain chromosome stability, will be presented.

GLOBAL REGULATION OF H2A.Z LOCALIZATION BY THE INO80 CHROMATIN REMODELING COMPLEX IS ESSENTIAL FOR GENOME INTEGRITY

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INO80 is an evolutionary conserved, ATP-dependent chromatin remodeling enzyme that plays key roles in many different DNA metabolic processes. Here, we show that the budding yeast INO80 complex regulates the genome-wide distribution of the histone variant H2A.Z. In wildtype cells, nucleosomes that contain the H2A.Z variant are enriched at discrete loci, including promoter regions for genes transcribed by RNA polymerase II. In the absence of Ino80, the amount of H2A.Z that is incorporated into chromatin is unchanged, but H2A.Z nucleosomes are more broadly distributed. Furthermore, H2A.Z levels at promoters show fewer changes in response to transcriptional status, suggesting decreased dynamics. Additionally, we demonstrate that purified INO80 complex has a novel histone exchange activity in which the enzyme can replace nucleosomal H2A.Z/H2B dimers with free H2A/H2B dimers in an ATP-dependent reaction. In contrast, SWI/SNF, RSC, and SWR1 enzymes are unable to perform this dimer exchange activity. To investigate the functional significance of H2A.Z mislocalization, we have tested whether mutations or depletion of H2A.Z alleviates phenotypes of an ino80 mutant. We show that depletion of H2A.Z allows ino80 cells to progress through S phase in the presence of replication stress. Furthermore, we find that glutamine substitutions of four lysine residues within the H2A.Z N-terminal domain alleviates the sensitivity of ino80 mutants to both replication stress and DNA damaging agents. We propose that the removal and replacement of unacetylated H2A.Z from chromatin is an essential and novel mechanism by which INO80 maintains genome integrity.

MOLECULAR MECHANISMS OF PLURIPOTENCY AND REPROGRAMMING

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The recent demonstration of in vitro reprogramming using transduction of 4 transcription factors by Yamanaka and colleagues represents a major advance in the field. However, major questions regarding the mechanism of in vitro reprogramming need to be understood and will be one focus of the talk.

Direct reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) can be achieved by over-expression of Oct4, Sox2, Klf4 and c-Myc transcription factors, but only a minority of donor somatic cells can be reprogrammed to pluripotency. We have demonstrated that reprogramming is a continuous stochastic process where almost all donor cells eventually give rise to iPSCs upon continued growth and transcription factor expression. Inhibition of the p53/p21 pathway or over expression of Lin28 increased the cell division rate and resulted in an accelerated kinetics of iPSC formation that was directly proportional to the increase in cell proliferation. These results suggest that the number of cell divisions is a key parameter driving epigenetic reprogramming to pluripotency. In contrast, Nanog over expression accelerated reprogramming in a predominantly cell division rate independent manner.

A major impediment in realizing the potential of ES and iPS cells to study human diseases is the inefficiency of gene targeting. Using Zn finger mediated genome editing we have established efficient protocols to target expressed and silent genes in human ES and iPS cells. Finally, our progress in using iPS cells for therapy and for the study of complex human diseases will be summarized.

RESETTING THE MOUSE EPIGENOME IN GERM CELLS FOR TOTIPOTENCY

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A primary role of the germ cell lineage is to generate the totipotent state. In mice this entails resetting the epigenome through genome-wide active DNA demethylation and extensive chromatin modifications in early germ cells. Primordial germ cells (PGC) in mice originate from postimplantation epiblast cells that have already initiated the somatic program. This trend is arrested in PGC precursors through the expression of key germ cell determinants: Prmt5, Lin28, Blimp1/Prdm1 and Prdm14. This is accompanied by epigenetic modifications, which erase the epigenetic memory of their initial trajectory towards somatic fate that is replaced by re-expression of key pluripotency genes and initiation of germ cell-specific program. Further extensive and genome-wide epigenetic changes occur when PGCs migrate into the developing genital ridge. At about E11.25, PGCs exhibit extensive changes over a period of 4-6h, independently of DNA replication and when cells are in the G2 phase of the cell cycle. This includes changes in nuclear architecture at the time of active DNA demethylation, followed by extensive chromatin remodelling and histone replacement through histone chaperones, NAP-1 and HIRA. Active DNA demethylation is mechanistically linked to generation of single-strand DNA breaks involving the base excision DNA repair pathway. The overall epigenetic changes, including DNA demethylation and chromatin modifications are crucial for resetting the epigenome for totipotency. Deeper understanding of these events may allow recapitulation of these events in in vitro systems.

ESTABLISHING GENETIC COMPETENCE FOR CHROMATIN IN DEVELOPMENT AND MITOSIS.

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Our laboratory is interested in the molecular mechanisms by which transcription factors and chromatin states endow the competence for multipotent progenitors to activate genes of different cell fates. The means by which genes become competent for developmental expression may be recapitulated as cells enter mitosis, when active chromatin becomes silent but must be re-activated faithfully during mitotic exit. As a model system, we investigate how the endoderm germ layer in mouse embryos becomes competent to activate the genetic programs for liver and pancreas cells, and once those cell fates are selected, how the genetic programs are maintained through mitosis. We originally found that FoxA and GATA factor binding sites are occupied at a silent liver gene in undifferentiated endoderm cells and that FoxA factors can expose a local nucleosome, thus allowing other factors to enter the chromatin, when they are appear during cell differentiation. Recently, we found that silent liver genes in undifferentiated endoderm cells have a pattern of epigenetic marks that is distinct from that seen at silent pancreas genes in the same cells, despite that both liver and pancreas genes are competent for activation. Using genetics and pharmacological approaches, we have further shown the marks to be functional. The different states of competence for the activation of different tissue programs in a single multipotent progenitor, as seen in embryos, presents a benchmark by which it may be possible to predict the developmental competence of stem cells and in tissue regeneration. We also found that FoxA factors remain engaged in chromatin through mitosis. The mechanism and function of pioneer factor engagement in mitotic chromatin and the involvement of other proteins in mitotic complexes will be discusssed.

CONNECTING TRANSCRIPTIONAL CONTROL TO CHROMOSOME STRUCTURE AND HUMAN DISEASE

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Discovering how transcriptional regulatory circuitry and chromatin structure control gene expression programs in mammalian cells is important for understanding the control of cell state, the process of development and the mechanisms involved in certain diseases. Using shRNA screens, we have identified novel transcriptional and chromatin regulators that contribute to the control of embryonic stem cells and induced pluripotent stem cells. I will describe new insights from our studies of these regulators that connect cell-type specific transcriptional regulation to chromosome structure and human disease.

DECIPHERING AND RECONSTRUCTING THE EMBRYONIC STEM CELL TRANSCRIPTIONAL REGULATORY NETWORK

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Embryonic stem (ES) cells are characterized by their ability to self-renew and remain pluripotent. Transcription factors have critical roles in the maintenance of ES cells through specifying an ES-cell-specific gene expression program. Deciphering the transcriptional regulatory network that describes the specific interactions of these transcription factors with the genomic template is crucial for understanding the design and key components of this network. To gain insights into the transcriptional regulatory networks in ES cells, we use chromatin immunoprecipitation coupled to ultra-high-throughput DNA sequencing (ChIP-seq) to map the locations of sequence specific transcription factors. These factors are known to play different roles in ES cell biology. Our study provides new insights into the integration of these regulators to the ES cell-specific transcription circuitries. Collectively, the mapping of transcription factor binding sites identifies new features of the transcriptional regulatory networks that define ES cell identity. Using this knowledge, we investigate nodes in the network which when activated, will jump-start the ES cell-specific expression program in somatic cells.

CHANGES IN CHROMATIN FIBRE DENSITY AS A MARKER FOR PLURIPOTENCY

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An open chromatin architecture devoid of compact chromatin is thought to be associated with pluripotency in embryonic stem cells. Establishing this distinct epigenetic state may also be required for somatic cell reprogramming. However, there has been little direct examination of changes in global structural domains of chromatin during the founding and loss of pluripotency. We have used Electron Spectroscopic Imaging (ESI) to examine large-scale chromatin structural changes during the transition from one-cell to early postimplantation stage mouse embryos. In one-cell embryos chromatin is extensively dispersed with no noticeable accumulation at the nuclear envelope. In eight-cell embryos and pluripotent epiblast cells, chromatin is primarily distributed as an extended meshwork of uncompacted fibres and is indistinguishable from chromatin organization in embryonic stem (ES) cells. In contrast, lineage-committed trophectoderm and primitive endoderm cells, and the stem cell lines derived from these tissues, display higher levels of chromatin compaction, suggesting an association between developmental potential and chromatin organisation. We found that deletion of Oct4, a factor required for pluripotency, causes the formation of large blocks of compact chromatin in putative epiblast cells. Together, these studies show that an open chromatin architecture is established in the embryonic lineages during development and is sufficient to distinguish pluripotent cells from tissue-restricted progenitor cells. Large scale chromatin reorganization also accompanies reprogramming of induced pluripotent stem cells (iPSC), an epigenetic process that reactivates the pluripotent transcriptional network by erasing or establishing heterochromatin marks. We have characterized the physical structure of heterochromatin domains in full and partial mouse iPSCs by correlative ESI. We show that in somatic cells and partial iPSCs, H3K9me3 marked constitutive heterochromatin is highly compartmentalized into chromocentre structures of densely packed 10 nm chromatin fibres. In contrast, chromocentre boundaries are poorly defined in pluripotent ESCs and full iPSCs due to a nanog dependent dispersal of the 10 nm heterochromatin fibre density. This heterochromatin reorganization accompanies retroviral silencing during conversion of partial iPSCs by Mek/Gsk3 2i inhibitor treatment. We conclude that constitutive heterochromatin structure is largely composed of 10 nm fibres, and its dispersal serves as a biomarker for very late stages of reprogramming.

IN VITRO REPROGRAMMING OF SOMATIC CELLS TO CANCER STEM CELLS

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Cancer stem cells (CSCs) have been implicated in the maintenance and progression of several types of cancer. The cellular origin of putative CSCs is unclear. They may arise by transformation of normal stem cells or alternatively by de-differentiation of mutated somatic cells. As part of our efforts to understand tumor resistance in patients with the premature aging laminopathy Hutchinson-Gilford Progeria Syndrome, we have characterized the cellular properties of cancer-inducing cells generated during in-vitro transformation. We find that human somatic cells can be reprogrammed by in vitro transformation to generate cells with hallmarks of CSCs. Transformation confers stem cell properties to primary differentiated fibroblasts, including their ability to self-renewal and pluripotency. Expression of a cell surface marker prospectively identifies a subpopulation of transformed cells responsible for tumour development. In vivo lineage-tracking experiments demonstrate hierarchical development of the tumor mass from stem-cell-like transformed fibroblasts and show that heterogeneous lineages of cancer cells arise by differentiation of reprogrammed fibroblasts. Our results demonstrate that transformed somatic cells can de-differentiate to acquire properties of cancer stem cells and act as cells of origin of cancer.

CYTOPLASMIC TAIL OF EGFR LIGAND, AMPHIREGULIN REGULATES HISTONE MODIFICATION AND INDUCES THE CELL MIGRATION

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Epiedermal growth factor (EGF) receptor ligands are synthesized as type I transmembrane precursors (pro-form) and express on the cell surface. The pro-form comprises an extracellular EGF-like domain, a transmembrane segment, and a short cytoplasmic tail. The ectodomain shedding yields a carboxyl-terminal fragment containing transmembrane and cytoplasmic tail as well as a soluble EGF receptor (EGFR) ligand. The EGF-like domain alone is sufficient to elicit EGF receptor phosphorylation on distant cells, neighbor cells, or on the cell of its origin. Conversely recent studies have shown biological activities for the conserved cytoplasmic tail of EGF family members. For instance, membrane anchored HB-EGF-cytoplasmic tails are localized at the inner nuclear membrane (INM) (Hieda et al., 2008) and regulate gene expression (Nanba et al., 2003; Kinugasa et al., 2007). Moreover proamphiregulin is also targeted to the INM and induces transient genome wide transcriptional repression (Isokane et al., 2008). Amphiregulin (AREG) has been established as an important paracrine mediator of normal ductal development in the mouse mammary gland and more specifically as a regulator of proliferation. The potential role of AREG in breast cancer was highlighted by the finding that the frequency and the level of AREG protein expression are generally higher in invasive breast carcinomas than in ductal carcinomas in situ or in normal mammary epithelium. AREG has been intensively investigated as a major paracrine and autocrine regulator via its extracellular EGF-like domain in cancer cells. In contrast, the role of its cytoplasmic tail in malignant cells is still unclear. To reveal the potential role of proAREG cytoplasmic tail, we used a proAREG mutant (here we termed Mu-proAREG). This mutant was localized at the ER and nuclear envelope without any stimuli and suppresses global gene expression i.e., mimicking the function of INM localized proAREG cytoplasmic tail. In this study we found that Mu-proAREG expression in a several breast cancer cell lines suppressed cell growth and activated cell motility. However, exogenously added soluble AREG (which binds and activates EGF receptor) did not suppress cell growth nor activate cell motility in these cell lines. Moreover Mu-proAREG expression induced the metylation of histone tails at H3Lys9. These results suggested a potential role for the cytoplasmic tail of proAREG in regulation of histone modification and cell motility. At moment we are trying to dissect the molecular basis for the histone modification and activated cell motility.

RANGAP1-REGULATED CYTOPLASMIC DIFFUSION OF NUCLEAR PROTEINS DURING MEIOSIS IN FISSION YEAST

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Meiosis is a process of general importance for sexually-reproducing eukaryotic organisms to produce inheritable haploid gametes from parental diploid cells. This process involves one round of DNA replication followed by two consecutive nuclear divisions (meiosis I and II). Thus, meiosis II proceeds without DNA replication, and meiosis-specific regulations must be involved. We have been studying nuclear events during meiosis in the fission yeast Schizosaccharomyces pombe, and noticed that nuclear proteins diffused to the cytoplasm transiently for a few minutes at onset of anaphase of meiosis II whereas such cytoplasmic diffusion never occurred in the mitotic cell cycle. We confirmed that nuclear envelope and NPC components remain intact throughout the mitotic cell cycle as well as in meiosis, by using a method of live CLEM (correlative light and electron microscopy after live imaging, in which we first follow GFP fusion proteins in living cells, and the same cells are subjected to electron microscopy for high-resolution imaging). Thus, this cytoplasmic diffusion of nuclear proteins is regulated with the intact nuclear envelope. Interestingly, this meiosis-specific cytoplasmic diffusion of nuclear proteins was not observed in mutants defective in sporulation, suggesting that this event may be required for sporulation. We found that this cytoplasmic diffusion of nuclear proteins was accompanied by nuclear translocation of GFP-fused Rna1, a RanGAP1 homologue in S. pombe. In addition, expression of GFP-Rna1 fused with nuclear localization signal (NLS) induced cytoplasmic diffusion of nuclear proteins in mitotic cells as well as meiotic cells, whereas as a control an enzymatically-inactive mutant of GFP-Rna1-NLS did not. These results suggest that the meiotic nuclear protein diffusion is regulated by the activity of Ran, a small GTP binding protein, through translocation of RanGAP. This RanGAP1-regulated cytoplasmic diffusion of nuclear proteins provides a novel way of shuffling protein components by reversing the nucleo-cytoplasmic transport in the presence of intact nuclear envelope, and may play an important role in production of gametes for organisms that undergo closed mitosis.

EFFICIENT TARGETING OF EXPRESSED AND SILENT GENES IN HUMAN ESCS AND IPSCS USING ZINC-FINGER NUCLEASES

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Realizing the full potential of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) requires efficient methods for genetic modification. However, techniques to generate cell type-specific lineage reporters, as well as reliable tools to disrupt, repair or overexpress genes by gene targeting, are inefficient at best and thus are not routinely used. We recently reported on the highly efficient targeting of three genes in human pluripotent cells using zinc-finger nuclease (ZFN)-mediated genome editing. First we generated OCT4-eGFP reporter cells to monitor the pluripotent state of hESCs by integrating a splice acceptor-GFP into the first intron of the OCT4 gene. Second, we inserted a transgene into the AAVS1 locus to generate a robust drug-inducible overexpression system in hESCs. Finally, we targeted the PITX3 gene, demonstrating that ZFNs can be used to generate reporter cells by targeting non-expressed genes in hESCs and hiPSCs. These three targeting events were achieved by the integration of a single selectable targeting cassette precisely at the site of the ZFN cut. Here we report that ZFN-mediated targeting can be used alter and remove genomic sequences in proximity of the ZFN cut. We illustrate this by targeting the OCT4 locus using a construct that generates a direct OCT4-GFP fusion protein by resection of the genomic intron sequence of the OCT4 locus. This finding adds to the general utility of a given ZFN pair by increasing the type and specificity of genetic manipulations it can facilitate in hESCs. Furthermore, we present data on the generation of transheterozygous clones by simultaneous one step targeting of both AAVS1 alleles with two different targeting cassettes in hESCs.

EMERIN BINDING TO LMO7 INHIBITS LMO7 BINDING TO THE PAX3 AND MYOD PROMOTERS AND EXPRESSION OF CRITICAL MYOBLAST DIFFERENTIATION GENES.

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Mutations in the gene encoding emerin cause X-linked Emery-Dreifuss muscular dystrophy (X-EDMD). Recent evidence suggests the muscle phenotype is caused by defective myogenic differentiation and muscle regeneration. Lmo7 was previously identified as an emerin-binding transcription activator that regulates a number of muscle genes. We report here that Lmo7 regulates myoblast differentiation by regulating the expression of critical myoblast proliferation and differentiation genes, including Pax3, Pax7, Myf5 and MyoD. Lmo7 associates with these promoters in myoblasts and binds directly to the promoters of Pax3 and MyoD in vitro. Emerin was previously predicted to inhibit transcription regulator activity by binding transcription regulators at the nuclear envelope and blocking transcription factor binding to their promoters. Here we directly tested this model and report that emerin binding to Lmo7 inhibits Lmo7 binding to Pax3 and MyoD promoters. We conclude that the physiological interaction between emerin and Lmo7 is critical for myogenic differentiation and muscle regeneration. These data further suggest that loss of emerin in X-EDMD might lead to muscle regeneration defects by causing aberrant temporal activation of myoblast differentiation genes by failing to regulate Lmo7 activity.

NPL3 BINDS NON-CANONICAL POLYA STRETCHES ON DIVERSE RNAS

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The SR protein, Npl3, is a yeast RNA binding protein that is implicated in multiple steps in mRNA processing including pre-mRNA splicing, 3’ end formation and mRNA export. It is unclear whether Npl3 is directly involved in all these processes, or whether a single function of Npl3 results in a multitude of phenotypes when *NPL3* is mutated/deleted. To understand the specificity of Npl3, we employed a UV cross-linking and sequencing strategy (CRAC) to identify all transcripts associated with Npl3 *in vivo*, and, importantly, *where* in these transcripts Npl3 is bound. Surprisingly we found that Npl3 binds to diverse RNA species that are polyadenylated at non-canonical sites; these RNAs include mRNAs, rRNAs, tRNAs, intergenically transcribed RNAs and RNA sequences running antisense to known transcripts. Several recent studies have demonstrated a role for the TRAMP complex, which includes a non-canonical poly-adenosine (polyA) polymerase Trf4/Trf5, in the degradation and/or processing of many RNA species. Our cross-linking data reveal that Npl3 binds sequences predicted to be degradation intermediates precisely across the junction between the 3’ end of the encoded RNA fragment and the start of the polyA sequence, suggesting an additional function for Npl3 in RNA stability. Additionally, several cryptic unstable transcripts (CUTs) that are known to be subject to TRAMP-mediated degradation are elevated in a strain in which *NPL3* is deleted, further suggesting a functional role for Npl3 in RNA turnover. Current experiments are directed towards elucidating the total range of polyadenylated transcripts bound by Npl3, the location within the sequences to which Npl3 binds, and the functional consequence of this binding.

ROLES OF COMPLEXES OF NUCLEAR MYOSIN 1 AND LIPIDS IN THE CELL NUCLEUS

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Nuclear myosin 1 (NM1) is one of the myosins that were recently found in the cell nucleus. We demonstrated earlier that together with nuclear actin, both proteins are associated with rRNA genes and with RNA polymerase I during transcription. As NM1 has been shown to be necessary for transcription and chromatin remodeling, it is important to reveal the modes of its action in the nucleus.
PH and co-transfected cells with NM1and wild type PLCδPH or mutant PLCδPH. FRAP results showed that NM1 mobility increased when PIP2 was occupied by wild type PLCδ compared to mutant one. All these data indicated that NM1 binds to PIP2 in the cell nucleus, and this was further confirmed by electron microscopy. The functional meaning of these complexes will be discussed.δNM1 is identical to its splicing varient Myosin 1C with the exception that NM1 has 16-residue N-terminal extension. It was shown that Myosin 1C binds to negatively charged phospholipids specifically to phosphatidylinositol(4,5)bisphosphate (PIP2) with a very high affinity (Hokanson et al. 2006) and this binding tethers NM1 to plasma membrane. Based on these findings we asked the question if NM1 also has binding properties to PIP2. In order to investigate this question we made single point mutations in the pleckstrin homology (PH) domain of NM1 where was shown to be responsible for PIP2 binding by Hokanson et al. and applied fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS). Mutant NM1 became faster in mobility compared to its wild type NM1 showing that NM1 bound to PIP2 and this binding slowed down the NM1 mobility. Then we depleted PIP2 in the nucleus by co-transfecting the cells with inositol 5-phosphatase which would cleave 5-phosphate of PIP2 in the nucleus and with NM1. When PIP2 was depleted by inositol 5-phosphatase NM1 became faster showing once more that PIP2 binding reduced NM1 mobility. Phospholipase C delta (PLCδ) is an enzyme binds to PIP2 via its PH domain and cleaves PIP2 into inositol (1,4,5) triphosphate and DAG. We mutated the PIP2 binding domain of PLC

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ROLE OF CTCF IN REGULATING DIFFERENTIAL CHROMATIN AT THE DIRAS3 LOCUS

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Imprinted genes are monoallelically expressed in a parent-of-origin dependent way. They are epigenetically regulated by allele specific DNA methylation, histone modifications and differential transcription factor binding.

DIRAS3 is a paternally expressed tumour suppressor gene coding for a ras homolog with growth suppressor activity. DIRAS3 undergoes loss of expression in various cancer types including breast, ovarian, pancreatic, oligodendrogliomas and ovarian cancers.

Quantitative analysis of DNA methylation shows that DIRAS3 has two differentially methylated regions, including the promoter, which are differentially methylated in all tissues tested. Methylation levels are shown to increase in cancer samples and cancer cell lines.

CTCF is known to regulate imprinted gene expression and is also sensitive to DNA methylation. CTCF has previously been shown to regulate higher order chromatin structures at other imprinted loci. To further investigate the function of differential methylation the binding sites of CTCF were identified. CTCF is shown to bind monoallelically at the promoter of DIRAS3. At the DIRAS3 region CTCF mediates chromatin looping which brings the DIRAS3 promoter on the active paternal allele in contact with an enhancer region. The maternal allele lacks CTCF binding and therefore does not associate with the enhancer. Methylation of the CTCF binding site in cancer cell lines prevents CTCF binding. Treatment with demethylating agents restores CTCF binding and DIRAS3 expression. This work has important implications for understanding the regulation of DIRAS3, which is silenced in many cancers.

DYNAMICS AND FUNCTION OF THE H3K27 DEMETHYLASE JMJD3.

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Gene silencing by the Polycomb group of proteins involves methylation of histone H3 lysine 27 (H3K27). A group of H3K27 demethylases has been identified which lead to a decrease of H3K27 methylation of genes activated upon cellular differentiation or exogenous signals. We provide a live cell analysis of the H3K27 demethylase JMJD3 in human cells. In addition to a diffuse nuclear pool, endogenous or YFP-tagged JMJD3 protein localizes to 20-40 nuclear foci, which are 0.2-0.5 μm in diameter. In interphase, JMJD3 foci co-localize with heterochromatin rich in H3K9me3, HP1α, HP1β, and HP1γ. During mitosis, the foci disassemble in prometaphase, and they are formed once again in early G1. A mutational and deletion analysis indicates that the localization of JMJD3 in nuclear foci depends on an intact N-terminus and Zn-finger domain. The diffuse, and foci associated JMJD3 can be distinguished by their mobility in a fluorescence recovery after photobleaching (FRAP) experiment. JMJD3 residing in foci has a significantly lower mobility than the diffuse pool, and the lower mobility depends on an intact JmjC domain. Interestingly, JMJD3 gets rapidly (within 3 min) and transiently recruited to a reporter gene upon transcriptional activation. Our data indicate a function of JMJD3 in transcriptional activation and/or chromatin remodeling. We are currently investigating the role of JMJD3 in transcriptional induction of endogenous genes.

NOVEL ROX2 RNA INTERACTING PROTEINS AND DOSAGE COMPENSATION

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Most animals determine their sex by means of an alternate inheritance of a pair of chromosomes, which are called the sex chromosomes. Usually one of these chromosomes is a degenerated version of its once homologous pair and this degeneration leaves one of the sexes with a single functional sex chromosome whereas the rest of the chromosomes come in pairs. Gene regulation is a tightly regulated process and such chromosomal differences between different sexes can and usually do create gene dosage problems which are addressed by a process called dosage compensation.

In mammals, females inherit two X chromosomes and males inherit one X and the degenerate Y chromosome. In order to equalize gene expression, females shut off one of their X chromosomes that is known thereafter as the Barr body. Flies on the other hand use a different strategy and males in this case transcriptionally hyperactivate their single X chromosome approximately two-fold. This process is carried out by the Male Specific Lethal (MSL) complex, which is composed of five proteins; MSL1, MSL2, MSL3, MOF and MLE, and two noncoding RNAs; roX1 and roX2. The MSL complex is targeted to and coats the male X chromosome almost entirely, and it has been proposed that roX RNAs might be important in this targeting.

roX RNAs are integral part of the dosage compensation complex, however very little is known about their function. We have taken a biochemical approach to study the function of roX RNAs and have identified novel roX interacting proteins. The progress of this project will be discussed.

TWO-STEP COLOCALIZATION MECHANISM OF MORC3 WITH PML-NUCLEAR BODY

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Many functional subdomains, including promyelocytic leukemia (PML)-nuclear bodies (NBs), are formed in the mammalian nucleus. Various proteins are constitutively or transiently accumulated in PML-NBs in a PML-dependent manner. MORC3 (Microrchidia family CW-type zinc finger 3), also known as NXP2, which consists of GHL-ATPase, a CW-type zinc finger and coiled-coil domains, is localized in PML-NBs, recruits p53 into PML-NBs, and activates p53 to induce cellular senescence. Interestingly, we found that MORC3 can form PML-independent nuclear domains (NDs) in mouse hematopoietic cells and even in Pml-deficient cells. Here we show that MORC3 colocalizes with PML via two-step molecular mechanisms: the PML-independent formation of MORC3-NDs by the ATPase cycle, and the association of MORC3 with PML via the SUMO1-SUMO interacting motif (SIM). Like other members of the GHL-ATPase family, MORC3 functions as a “molecular clamp”. ATP-binding induces conformational changes in MORC3, leading to the formation of MORC3-NDs, and subsequent ATP-hydrolysis mediates the diffusion and binding of MORC3 to the nuclear matrix. MORC3 may clamp DNA or nucleosomes in MORC3-NDs via the CW domain. Furthermore, the SUMOylation of MORC3 at five sites was involved in the association of MORC3 with PML, and SUMO1-unmodified MORC3 formed NDs independent of PML. Finally, we observed that in living cells, mEGFP-MORC3 dispersed by ATP-depletion and reformed ND in PML-NB-independent region by ATP-recovery, and that the ND fused to PML-NB.

STRESS-INDUCED ASSEMBLY OF DAXX/ATRX COMPLEX AT CENTROMERES IS SUMO-2/3 DEPENDENT

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Conjugation of target proteins to the small ubiquitin-like modifier SUMO-2/3 is required to the cell survival upon application of stress, including heat shock (HS). Despite description of numerous stress-induced SUMOylated targets, the specific consequences of this modification remain mostly elusive.
Here we describe stress-induced accumulation of protein Daxx at centromeres in human cells. Daxx, that is mostly resided at PML NB's/ND10 in control conditions, becomes accumulated at centromeres upon application of mild HS (42 C). While accumulation at PML NB's requires SUMO-1 and SUMO2/3, the mechanism of Daxx deposition at centromeres involves modification by SUMO-2/3, but not SUMO-1. It also requires functional Daxx SIM's, suggesting potential interaction at centromeres with SUMOylated partner. This accumulation can be observed already at 30 minutes of HS, is reversed 1 hour after HS release and does not involve changes in Daxx protein stability. Centromere distribution of Daxx can be also induced upon the inhibition of the proteosome-dependent protein degradation by MG132 treatment suggesting either degradation or de-SUMOylation of putative Daxx-interacting centromere partner as the potential mechanism of post-stress release of Daxx. While dynamics of Daxx accumulation is similar throughout interphase upon short HS application, the extension of HS results in longer retention of Daxx at centromeres in S/G2 compared to G1 phase, suggesting cell cycle dependent response to HS.
Among others tested PML NB's associated proteins, only Daxx interaction protein ATRX (alpha thalassemia/mental retardation syndrome X-linked chromatin remodeler) can follow Daxx re-positioning upon HS. Moreover, both SUMO-2/3 and Daxx are required to ATRX accumulation at centromeres, suggesting formation of stress-induced Daxx-dependent chromatin remodeling complex at these intranuclear structures. The physiological consequences of stress-induced Daxx/ATRX distribution will be discussed.

CENTROMERIC LOCALIZATION OF DISPERSED POL III GENES CONTRIBUTES TO GLOBAL GENOME ORGANIZATION AND CHROMOSOME CONDENSATION

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The eukaryotic genome is a complex three-dimensional entity residing in the nucleus. We present evidence that Pol III transcribed genes such as *tRNA* and *5S rRNA* genes can contribute to a global genome organization in fission yeast *Schizosaccharomyces pombe*. We find that Pol III transcription machinery, TFIIIB and Pol III, co-localize with centromeres, and that many Pol III genes dispersed throughout the genome localize in the vicinity of centromeres. Moreover, ectopic insertion of Pol III genes into a non-Pol III gene locus results in the centromeric localization of the locus, indicating that Pol III genes are a critical driver for the centromeric localization of genomic locus. We show that the centromeric localization of Pol III genes is mediated by condensin complex and is interfered with Pol III transcription. Remarkably, defective mitotic chromosome condensation by a condensin mutation, *cut3-477*, which reduces the centromeric localization of Pol III genes, is suppressed by a mutation in the *sfc3* gene encoding the Pol III transcription factor TFIIIC subunit, *sfc3-1*. The *sfc3-1* mutation reduces Pol III transcription. In the *sfc3-1* mutant, binding of the condensin to the Pol III gene is increased and chromosome condensation is promoted. Our study suggests there are functional links between process of the centromeric localization of dispersed Pol III genes, their transcription, and the assembly of condensed mitotic chromosomes.

STUDYING NUCLEAR STRUCTURE BY ATOMIC FORCE MICROSCOPY

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We have been analyzing the nuclear structure with the atomic force microscopy as an attempt to test the possibility of obtaining high resolution while observing under physiological conditions. Our approach has consisted in preparing samples as for transmission electron microscopy and use sections of that material placed onto glass slides to observed internal cell structure. We have been able to visualize well known nuclear territories from several biological systems like plant and animal cells. Compact chromatin, nuclear envelope and pores, and nucleoli are well defined. Here we show images of the nucleoplasm from cultured mammalian cells (HeLa or Hep2) at high vertical resolution. In addition, profiles of the texture of the nucleoplasm reveal regular arrangements that may be used to identify nuclear compartments as interchromatin granule clusters. In plant samples as onion (*Allium cepa*), nucleolar organizers and prenucleolar bodies during nucleologenesis at telophase are also observed. In addition, fibrous and granular elements are visualized within the nucleoli. Further studies will focus on changes of nuclear and nucleolar structure under different environmental and physiological conditions in different samples including, parasites as *Giardia lamblia* (DGAPA-UNAM IN227810).

THE SR PROTEIN B52/SRP55 RECRUITS DNA TOPOISOMERASE I DURING TRANSCRIPTION TO PROMOTE MRNA RELEASE AND TRANSCRIPTION SHUTDOWN

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Coupling between RNA transcription and splicing has emerged as a new level of regulation of gene expression, since transcription speed can affect splicing and reciprocally splicing factors, such as SR proteins, can influence transcription elongation. Among the proteins that lies at the boundary between transcription and splicing is the DNA topoisomerase I (TopoI). Indeed mammalian TopoI carries two enzymatic activities: a DNA topoisomerase activity that relaxes topological constraints during transcription and a kinase activity directed against the SR proteins family of splicing factors.
To get an insight into TopoI function *in vivo*, we have investigated the relationships between TopoI and the SR protein B52/SRp55 in *Drosophila*. We show that the kinase activity of TopoI is conserved in *Drosophila* and that TopoI phosphorylates B52 *in vivo*. Analysis of TopoI and B52 distribution on polytene chromosomes reveals a striking colocalization of the two proteins. We demonstrate that B52 influences TopoI localization and that TopoI recruitment on chromatin involves the B52 protein. As a model we used the *hsp70* gene on which TopoI and B52 are massively recruited after induction by heat shock. In the absence of B52, TopoI recruitment is compromised and *hsp70* mRNA accumulates at its transcription sites. Since *hsp70* is not spliced, this result reveals a new, splicing-independent, role of B52 in RNA release from transcription sites. Remarkably, during the recovery phase after heat shock, *hsp70* shutdown is dramatically delayed in *B52*$^{-}$ as well as in *topoI*$^{-}$ mutant backgrounds, revealing a defect in gene deactivation. These results show that TopoI recruitment through B52 allows efficient RNA release from transcription sites and is necessary for transcription shutdown.

LINKER HISTONE H1 ACETYLATION ADDS TO THE COMPLEXITY OF CHROMATIN MODIFICATIONS.

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In the recent years significant progress has been made in understanding the role of core histone modifications in transcriptional regulation and other DNA mediated processes. In those studies H1 has been an “overlooked” histone, in spite of being also subjected to multiple covalent modifications, including lysine acetylation. Histone acetyltransferases specific to H1 are not yet known and the function of those acetylation marks has not been determined. Taking into consideration the pivotal role of H1 in chromatin organization and dynamics, and the dramatic consequences of core histone acetylation on gene expression, it is important to unravel the role of H1 acetylation to better understand chromatin regulation.

In our study we functionally characterize for the first time the acetylation of a conserved lysine residue in the tail of H1.4. We determined *in vivo* and *in vitro* the enzymatic machinery targeting this site. Further we investigated its nuclear distribution by immunofluorescence and its genomic distribution by chromatin immunoprecipitation and ChIP-sequencing. Interestingly, we find it enriched at transcription start sites of active genes. This is the first study of a H1 modification genome-wide.

Elucidating the role of H1 modifications, such as acetylation, in the modulation of gene expression will elevate our understanding of the functionality of histone modifications to the higher level of chromatin organization.

H2B UBIQUITYLATION REGULATES NUCLEOSOME DYNAMICS AT REPLICATION FORKS

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Histone modifications are important determinants of gene expression, replication, DNA repair and cell cycle control. Ubiquitylated lysine 123 on H2B (H2Bub1) is shown to contribute to efficient reassembly of nucleosomes during RNA polymerase II (Pol-II)-mediated transcription elongation in yeast. Here, we demonstrate that absence of H2Bub1 (htb1-K123R mutant) causes a slowdown G1/S transition and a prolonged S phase as the initiation of nascent DNA synthesis is defective. Significantly, the cell cycle control of H2Bub1 is independent of H3K4 and K79 methylations, the two putative downstream signals of H2Bub1. Moreover, the altered S phase progression is not a result of checkpoint activation but caused by distorted nucleosome stability at the replication fork. Genetic analysis suggests that H2Bub1 functions as an intrinsic cell cycle regulator in parallel with replication factors, Sgs1 and Mrc1. Deletion of histone chaperones Asf1 deteriorates the growth of htb1-K123R cell under replication stresses but the synergistic phenotypes are not observed when combined with the deletions of Cac1, a histone chaperone assembles newly synthesized histone onto replicated DNA. Thus, these findings suggest that H2Bub1 contributes to nucleosome dynamics at replication fork independent of Asf1.

GENOME-WIDE ANALYSIS OF DNA REPLICATION IN HUMAN CELLS

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DNA replication is a critical cell cycle event that results in an exact copy of the genome for each daughter cell. Origins of replication on the chromosomes are activated in a specific temporal order. Over the last few years we have defined (a) the time of replication and (b) hundreds of validated, relatively efficient origins, in the 1% of the genome selected for the ENCODE studies. Time of replication (TR50) was highly predictive of chromatin structure and was used to define domains of active and repressed chromatin in the genome. Origins of replication were discovered in three-fold excess relative to that predicted by single-fiber studies, suggesting that 2 out of 3 origins do not fire on a given DNA molecule in a single cell cycle. A significant portion of the early origins of replication were found to be in proximity to transcription start sites and regulatory factor binding regions (RFBR), and 80-90% were at or near ORC binding sites. There are, however, an excess of ORC binding sites relative to origins. In this study we have analyzed what happens to the replication program when progression of DNA replication is blocked with hydroxyurea (HU) to trigger the intra-S phase checkpoint. Only the early origins fire as cells enter S-phase in the presence of HU (n=178), however, these are accompanied by many new initiation sites also in early replicating parts of the genome that cluster in zones of ~12 kb in size. 75% of these new initiation events occur at or near RFBR, and ~40% of these RFBR contain an E2F or a c-Myc binding site. When the HU-induced checkpoint is abrogated by adding caffeine, 186 additional origins are activated and 35% of these arise in mid and late replicating chromosomal segments. A third of these sites show proximity to RFBRs. There was only a moderate effect of caffeine seen on replication elongation. To conclude, the time of replication appears to be dictated by activating/repressing marks on the chromosomes with significant plasticity in origin firing on a given DNA molecule. In keeping with this plasticity, replication stress leads to clustered firing of new origins near the stalled forks from normally used origins. Abrogation of the intra-S phase checkpoint in presence of HU initiates origins in later replicating parts of the genome but fails to complete S-phase due to lack dNTPs.

APPROACH TO PROFILE PROTEINS THAT RECOGNIZE POST-TRANSLATIONALLY MODIFIED HISTONE "TAILS"

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Post-translational modifications (PTMs) of histones, proteins onto which DNA is packaged, are involved in many biological processes, including transcription, recombination, and chromosome segregation. As these PTMs can be dynamic, combinatorial, and mediators of weak interactions, the comprehensive profiling of all proteins that recognize histone PTMs is a daunting task. Here we report an approach to design probes that can be used to identify proteins that directly interact with modified histones. Protein structure was used to guide the introduction of a photo-cross-linker in the probe, so as to convert weak interactions into covalent linkages. The probe also included an alkyne group to facilitate click chemistry-mediated conjugation of reporter tags for the rapid and sensitive detection (via rhodamine) and affinity enrichment (via biotin) of labeled proteins. First we confirmed that ING2, a known H3K4me3-binding protein, was specifically captured from HeLa cell extracts by H3K4me3 probe. Second, we extended this method to fission-yeast cells to check generality, and showed that Swi6 (fission-yeast homolog of HP1), a known H3K9me3-binding protein, was specifically captured from fission-yeast extracts by H3K9me3 probe. Coupling with proteomic analysis, we are now working on a comprehensive profiling of histone modification-recognition proteins using this approach.

MAPPING MARS IN 1% OF THE GENOME REVEALS DIFFERENT TYPES OF ATTACHMENT TO THE NUCLEAR MATRIX.

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Current models of nuclear architecture describe compact chromosome territories that are organized by the tethering of 50-200 Kb chromatin loops to a nuclear scaffold, referred to as the nuclear matrix. In this study we purified and hybridized nuclear matrix associated DNA from HeLa cells to the ENCODE genome tiling microarray to identify the location of matrix attachment regions (MARs) within 1% of the human genome. Surprisingly, 58% of the 618 sites indentified by microarray analysis fell into clusters of MARs that had an inter-MAR distance of 4 KB or less. After consolidation of these sites, we report 143 MAR clusters with a median size of 8474 bp and 259 nonclustered MARs with a median size of 2041 bp. A median inter-MAR distance of 50 Kb was observed for the 402 MARs distributed across 44 different genomic regions. Comparison with genomic features revealed that MAR clusters localized to genes (76%, $P<0.001$) and near transcription start sites (46%, $P<0.0001$) and were associated with gene expression. In contrast, individual MARs did not show statistically significant association with genes or transcription start sites but were enriched for origins of replication and insulator protein CTCF binding sites. Both classes of MARs were significantly associated with features of open chromatin, such as DNAseI hypersensitivity sites, histone H3 and H4 acetylation, and early replicating regions of the genome ($P<0.0001$). We propose that clustered and nonclustered MARs likely represent two different classes of attachment sites. Clustered MARs consist of sites associated with transcription factories located on the nuclear matrix, while nonclustered MARs contain functional elements such as replication origins, insulators, boundary elements, and structural attachments responsible for the organization of the genome into functional domains.

IDENTIFICATION OF CAJAL BODIES IN PIGEON (*COLUMBA LIVIA*) OOCYTE NUCLEUS WITH INACTIVATED NUCLEOLAR ORGANIZER

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Cajal bodies (CBs) that are considered to be evolutionary conserved nuclear organelles, take part in different intranuclear processes such as final steps of maturation of small nuclear (sn) and nucleolar (sno) RNAs. Despite CBs were described over 100 years ago, precise functions and molecular composition of these domains remain obscure. The investigation of existing types of functional nuclear organization *in vivo* broadens our knowledge about the role of CBs. Because of large size and peculiarities of organization (lack of any nucleoli) avian oocyte nucleus (germinal vesicle, GV) is an advantageous model for such analysis. In avian GVs containing transcriptionally active chromosomes in the lampbrush form CBs have not been identified yet. At the same time, in pigeon (*Columbia livia*) GV two types of extrachromosomal spherical bodies called «hollow spheres» (HSs) and «solid globes» (SGs) were earlier described.
In the present work we investigated the molecular composition and possible functions of these remarkable nuclear bodies as well as the 3D-architecture of pigeon GVs. Staining of isolated GVs with NA-specific fluorescent dye Sytox green and anti-DNA antibodies showed that HSs and SGs both contain RNA. Using confocal laser scanning microscopy we found that RNA-enriched spheres (from single entity to several dozens) irregularly localize within the interchromatin space of the pigeon GVs. Electron microscopy revealed that dense matrix of both HSs and SGs does not contain any granules and has fibrillar fine structure. Inside the GVs only HSs and SGs but not other intranuclear structures contain p80-coilin that is one of the marker components of CBs in a variety of species including birds. Further analysis of molecular composition of nuclear structures in pigeon GVs elucidated that extrachromosomal spheres also accumulate mature snRNA and snRNP core proteins, but do not contain splicing factor SC35, the latter accumulating in RNP-matrix of chromosomal loops. Therefore, in pigeon oocyte nucleus the analogues of CBs were identified. Notably, in pigeon GVs extrachromosomal CBs do not recruit nucleolar proteins namely Nopp140, NO38 and fibrillarin. The unusual molecular composition of CBs in pigeon GVs correlates with the absence of functional nucleoli in oocytes that confirms the postulated hypothesis of CB’s modular structure. Unique molecular composition of CBs in pigeon oocytes together with peculiarities of avian oogenesis allows suggesting that HSs and SGs participate in snRNA but not snoRNA biogenesis.
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AEBP2 AS A POTENTIAL EPIGENETIC MODIFIER FOR NEURAL CREST CELLS

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Aebp2 (adipocyte enhancer binding protein 2) is a transcription factor conserved from flying insects to mammals. According to our recent studies, Aebp2 is most likely a targeting protein for the mammalian Polycomb Repression Complex 2. However, the *in vivo* function of Aebp2 during the development of multi-cellular organisms is not known. We have generated a mutant mouse line using a gene trap clone, in which a *Neo-LacZ* fusion cassette has inserted and disrupted the transcription of the endogenous mouse *Aebp2* gene. Genetic breeding experiments revealed that the homozygotes for the *Aebp2*-mutant allele are lethal at the peri-implantation stage, but the heterozygotes are viable and fertile, confirming an essential role for Aebp2 during early embryogenesis. In developing mouse embryos, *Aebp2* is mainly expressed within the cells of the neural crest origin, such as the dorsal root ganglion, cranial facial cartilages, thymus and the outer layers of the heart, lung, and intestines. In addition, potential downstream genes of *Aebp2*, discovered through our ChIP-Seq experiments, were highly changed in the *Aebp2*-heterozygous mice. Overall, the *Aebp2* expression within the neural crest cells strongly suggests that Aebp2 may be an epigenetic modifier for the development of the neural crest cells.

INHIBITING SPLICING CAUSES ACCUMULATION OF H3K36ME3 AT THE 3'END OF A GENE.

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In eukaryotes, capping, splicing, and polyadenylation of mRNA occur co-transcriptionally. Co-transcriptional processing is coordinated by the c-terminus of RNA polymerase II, which serves as a landing pad for the processing factors at the site of transcription. How the recruitment and assembly of the large processing and transcription machinery occurs in the context of chromatin remains elusive. Recent cataloging of genome wide histone modifications revealed enrichment of nucleosomes and H3K36Me3 modification on exons, linking splicing with chromatin state. In order to understand how H3K36Me3 correlates with splicing, we have generated CHO cell lines expressing either wild type β-globin or β-globin with cis mutants that interfere with splicing. Using these cell lines, we performed ChIP analysis to determine how splicing mutations effect chromatin. We observed that deletions of the 3’ splice site in intron2 or in both intron 1 and 2, result in pol II termination defect and caused a concurrent decrease of nucleosomes at the 3’end of a gene. Furthermore, we detected retention of H3K36Me3 in the 3’end-flanking region. This hyper methylation of H3K36 is not due to termination defect because a mutation of the polyadenylation signal (A2GA3), which causes delayed termination, did not alter the pattern of H3K36Me3. Taken together, our result suggests that the act of splicing is linked to the metabolism of H3 K36 methylation.

THE CHROMOSOMAL LOCALIZATION OF CONDENSIN II IS REGULATED BY A NON-CATALYTIC ACTION OF PP2A

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The assembly of mitotic chromosomes in vertebrates is regulated by condensin I and condensin II, which work cooperatively but demonstrate different chromosomal localization profiles and make distinct mechanistic contributions to this process. We show here that protein phosphatase 2A (PP2A), which interacts with condensin II but not condensin I, plays an essential role in the chromosomal association of condensin II. Surprisingly, our data indicate that PP2A acts as a recruiter protein rather than a catalytic enzyme to localize condensin II to mitotic chromosomes. This recruiting activity of PP2A was inhibited by okadaic acid, but not by fostriecin, even though both molecules strongly inhibited the catalytic activity of PP2A. Additionally, we found that the chromokinesin KIF4a is also targeted to chromosomes via the non-catalytic activity of PP2A. Thus, our studies reveal a previously unknown contribution of PP2A to chromosome assembly.

IMAGING THE CELLULAR HISTORY OF GENOME - NUCLEAR LAMINA INTERACTIONS

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By employing the DamID technique our lab recently showed that the genome at the nuclear lamina (NL) is organized in large domains (LADs) that are sharply demarcated by specific sequence elements such as CTCF binding sites (Guelen et al, Nature 2008). Within this nuclear organization the genome at the NL is believed to form a scaffold for gene silencing as most genes within LADs are very lowly expressed. What is still unclear is (i) how stable the interactions of LADs with the NL are over time; (ii) whether in each individual cell only a subset of LADs interacts with the NL; (iii) whether after mitosis the same or a different subset associates with the NL. Here we present a new "history tracking" imaging technique to address these questions.
DamID mapping of NL interactions is based on the in vivo expression of a chimeric protein consisting of Dam –an adenine DNA methyl transferase from E. coli - fused to a NL protein such as LaminB1. This fusion protein is incorporated in the NL and will methylate adenines of any DNA that is in molecular contact with the NL. Adenine methylation (which does not occur endogenously in eukaryotes) is stable, and thus transient contact with the NL is sufficient to leave a long-term mark on the DNA. To visualize this mark in live cells we searched for a protein-domain that would bind to the adenine methyl mark. We found the desired domain, termed DPN7, within the C-terminal part of the restriction enzyme DpnI. When fused to a fluorescent protein, DPN7 proves to be a robust in vivo methyl-adenine marker. When co-expressed with Dam-LaminB1, DPN7-GFP is selectively localized in a thin layer of chromatin close to the NL; in contrast, co-expression with unfused Dam yields a dispersed DNA staining throughout the nucleus. This tracer system now allows live cell imaging of DNA that is- or has been in contact with the NL. Here we will present our first time-course and live-cell imaging results on the dynamics of genome-NL interactions. In addition, based on a small candidate screening approach, we show the potential of the system to identify new factors involved in the establishment and/or maintenance of these interactions.

IDENTIFICATION OF SIX NUCLEAR ENVELOPE PROTEINS THAT CAN INFLUENCE THE CELL CYCLE/ SIGNALING CASCADES

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During the last decade it became evident that specific nuclear envelope proteins are involved in cell cycle progression. Lamin A, Lap2α, and Emerin all contribute to cell cycle regulation via the retinoblastoma (Rb) signaling pathway. In several recent proteomic analyses, we identified a large number of novel nuclear envelope transmembrane proteins (NETs) of which many have no known function. We considered that some of these novel NETs might separately or concurrently contribute to cell cycle regulation. To test this we used FACS analysis to measure the cell cycle profiles of HEK293T cells transiently transfected with NETs fused to mRFP and comparing them to the profile of the internal control (non transfected cells) or mRFP-only transfected cells. Six out of 40 NETs tested showed accumulation of transfected cells in the G2/M peak (4N) and one increased the population of cells in the G1 peak (2N). Three of the seven NETs (Tmem53, NCLN and FAM3C) yielded a significant reduction in phospho-Rb staining when overexpresed. The four that did not affect Rb phosphorylation could alternatively mediate their cell cycle effect through p53, another master cell cycle regulator; however no change in cell cycle phenotype was observed when NETs were transiently transfected into p53 null cells. Tmem53 was chosen for more focused studies. FACS results were confirmed by counting the mitotic index and overexpression and knockdown of TMEM53 caused a decrease in Ki-67 staining, indicating a withdrawal from the cell cycle. Tmem53 protein levels vary during the cell cycle stages and are lowest in mitosis. We propose that reduction of Tmem53 levels facilitates mitosis, but Tmem53 is needed to maintain cycling cells and that its effects are mediated on the p53/Rb pathway.

THREE-DIMENSIONAL ORGANIZATION AND MOLECULAR COMPOSITION OF NUCLEAR DOMAINS, LAMPBRUSH CHROMOSOMES AND INDIVIDUAL TRANSCRIPTION UNITS IN AVIAN GROWING OOCYTES

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Nuclear compartmentalization is one of the hierarchical levels in regulation of gene expression. Determinant variations in nuclear organization in growing vertebrate oocytes represent an advantageous model to study the principles of nuclear structure and function. In the present work we studied the three-dimensional (3D) complexity and molecular composition of various nuclear structures in oocyte-follicle cell complexes of four avian species (domestic chicken, Japanese quail, pigeon and chaffinch). Intranuclear compartments in microsurgically isolated 3D-preserved oocyte nuclei (germinal vesicles, GVs) were revealed with specific fluorescent dyes or antibodies. Nascent transcripts of satellite repeats on individual transcription units of lampbrush chromosomes (LBCs) in a third dimension were visualized by 3D-FISH performed on non-deformed nucleus. 3D organization of LBCs and nuclear organelles was analyzed by confocal LSM and computational image processing procedures such as iso-surfacing and 3D-reconstruction. These approaches have provided extensive insight into the functional compartmentalization of avian oocyte nucleus.
Analysis of spatial organization of giant LBCs has demonstrated the existence of repulsion between brushes that occupy the inner part of the oocyte nucleus. In contrast to somatic cells, in avian oocytes gene-rich microchromosomes have no preferential localization within the nuclear interior. In all avian species studied, with the only exception to pigeon, GVs of adult females are characterized by the absence of any extrachromosomal Cajal bodies (CBs). Surprisingly, in contrast to somatic cells, in growing oocytes of chicken, histone locus bodies (HLBs) are not present. These results demonstrate that neither CB nor HLB formation is necessary in avian GV. At the same time nucleolated cells of follicular epithelium that surround avian oocytes comprise CBs and SC35-domains. We further discovered that a number of intranuclear domains characterized by enrichment with specific sets of RNPs represent individual transcription units of LBCs. Associated with splicing factors transcripts of novel satellite repeat LL2R are involved in the formation of SC35-domains in chicken GVs. Another example of nuclear domains structured by the non-coding transcripts of tandem repeats (short 41 bp repeats) is subcompartments enriched with proteins K/J or C1/C2 of hnRNPs.
A role of actin polymerisation in structural maintenance of genome architecture in avian and amphibian GVs was also investigated. We found that there is a continuous polymerization and depolymerization of actin oligomers within the nucleus of lampbrush stage oocyte. Reorganization of genome architecture during oocyte maturation and karyosphere formation is driven by depolymerization of short actin polymers inside GV.

SPECTRIN-REPEAT PROTEINS IN THE NUCLEUS: MOLECULAR MECHANISM AND BIOLOGICAL SIGNIFICANCE OF THE NUCLEAR LOCALIZATION

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Spectrin-repeat (SR) is among the most common repeated domains consisted of a bundle of three α-helixes found in large structural proteins such as α-actinins (102-105kDa), nesprins (799-1011kDa), dystrophin (427kDa) and spectrin itself (268-417kDa). Most of the SR proteins are dominantly localized to the plasma membrane and/or cytoskeleton and function in the physical maintenance of cell structure. In the last few years, several SR proteins were also found to be localized to the nucleus and involved in the stabilization of subnuclear compartments such as nuclear envelope and PML bodies. These proteins must be controlled by an as-yet-identified nuclear-localization mechanism, as most of them lack an obvious NLS in their amino acid sequences.

We have previously reported that GFP-fused SR region of α-actinin-4 was localized to the nucleus. In this study, we widely focused on several SR proteins such as α-actinin-1, spectrin and dystrophin to investigate the property of SR as a nuclear transporting region. Interestingly, when the fragments containing 3-6 tandem SRs were expressed in HeLa cells, all of them were found to be localized to the nucleus. Nuclear-import assay with semi-permeabilized cells showed that some of these fragments possess strong nuclear-import activity. Furthermore blocking of FG-Nups in the NPC by an importin-beta mutant effectively inhibited the nuclear import of the SR fragments, suggesting that SRs migrates through the NPC by interacting with FG-Nups.

To further analyze the function of SR proteins in the nucleus, we focused on α-actinin-4, which best resembles the ancestral proteins of the spectrin superfamily. When the cells for immunostaining were pre-treated with leptomycin B, an inhibitor for CRM1-dependent nuclear export, endogenous actinin-4 accumulated to the nucleus. Thus, actinin-4 serves as a nucleocytoplasmic shuttling molecule, and the nuclear import is expected to be mediated by an interaction between SR region and FG-Nups. Actinin-4 was co-precipitated with INO80 chromatin-remodeling complex, one of general transcriptional regulators. Quantitative PCR analysis revealed that a subset of INO80-regulated gene expressions was affected in actinin-4 knockdown cells, leading us to propose that actinin-4 functions as a link between cytoskeletal organization and gene expression.

Our findings uncovered a novel importin-independent nuclear-transport mechanism of structural proteins. Given their large size and multiple roles in cytoplasmic organizations, SR proteins are expected to run into the nucleus by themselves to play an important role in the regulation of nuclear functions.

THE PROTEIN PHOSPHATASE 1 REGULATOR PNUTS IS A NOVEL COMPONENT OF THE DNA DAMAGE RESPONSE

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PNUTS, one of the most abundant nuclear targeting subunits of protein phosphatase 1 (PP1c), has been previously involved in the regulation of apoptosis and retinoblastoma dephosphorylation in response to stress. We demonstrate here that PNUTS is a central element in the DNA damage response. Depletion of PNUTS results in prolonged mitotic prophase, consistent with activation of the G_2 checkpoint leading to a G_2/M delay. Accordingly, addition of checkpoint inhibitors leads to increased mitotic entry of PNUTS-depleted cells. PNUTS depletion also enhances G_2 arrest and prolongs activation of Chk1 following γ-radiation-induced DNA damage, whereas overexpression of PNUTS-EGFP inhibits G_2 arrest after γ-radiation. PNUTS-EGFP is recruited to sites of DNA double stranded breaks in a rapid and transient manner. Finally, recruitment of the double strand break marker γH2AX as well as of 53BP1, RPA and Rad51, are prolonged in PNUTS-depleted cells following γ-radiation-induced DNA damage. We identify the PP1c regulatory subunit PNUTS as a novel and integral component of the DNA damage response involved in DNA repair.

MECHANISM AND A PEPTIDE MOTIF FOR TARGETING PERIPHERAL PROTEINS TO THE YEAST INNER NUCLEAR MEMBRANE

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In *Saccharomyces cerevisiae*, the *TRM1* gene codes for the tRNA-specific N2, N2-dimethylguanosine methyltransferase found in mitochondria and nuclei. Two isomeric forms of the proteins, Trm1-I and Trm1-II, are encoded via alternative translation initiation sites. Trm1-I is exclusively localized to the mitochondria while Trm1-II is found primarily in the nucleus at the inner nuclear membrane (INM). Although it has been shown that Trm1-II is peripherally associated with the INM, the mechanism of targeting to the INM is unclear. Studies using mutations of the Ran pathway and nuclear pore components showed that Trm1-II accesses the nucleoplasm via the classical Ran-dependent nuclear import pathway. If nuclear entry is inhibited either by disrupting the Ran pathway or by altering the context of Trm1-II's nuclear localization sequence (NLS), then Trm1-II-GFP is delivered to mitochondria. The results indicate that the NLS within Trm1-II is in competition with mitochondrial targeting information. A study of Trm1 *cis*-acting sequence identified the region of amino acids 89-151, containing the endogenous NLS, to be sufficient for INM localization. To characterize the amino acids important for targeting endogenous Trm1-II to the INM, we employed random and site-directed mutagenesis of the coding sequence for amino acids 89-151, and uncovered specific amino acids necessary for authentic Trm1-II to locate at the INM. We defined a sequence of ~20 amino acids that contains information necessary to target Trm1-II to the INM. To address whether the newly defined region necessary for Trm1-II INM location is also sufficient to target reporter proteins to the INM, we provided the 20 amino acids motif to three different reporter proteins: NLS-β-galactosidase, NLS-GFP, and NLS-Trm7-GFP. The data showed that this short peptide causes the redistribution of all 3 reporter proteins from the nucleoplasm to the INM. Thus, we identified the first motif for targeting peripherally associated proteins to the INM in yeast.

CHARACTERIZING THE ROLE OF HISTONE H3-S10 AND S28 PHOSPHORYLATION IN TRANSCRIPTIONAL ACTIVATION

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Histone phosphorylation is often a direct outcome of activated intracellular signaling pathways, and functions to translate extracellular signals into appropriate biological outputs such as changes in gene expression. Growth factor stimulations and cellular stress trigger rapid and transient expression of immediate-early genes (*c-fo*s, *c-jun*) in mammalian cells, and their induction strongly correlates with a transient phosphorylation of S10 and S28 on histone H3. While many signal transduction cascades that lead to H3 phosphorylation have been mapped out, the mechanistic details of the downstream events and how H3 phosphorylation contributes to transcriptional activation are still poorly defined. Using the Gal4-targeting / luciferase reporter system, we examined the transcriptional consequences of direct targeting known H3 kinases (MSK1, RSK2) to a promoter. Surprisingly, MSK1, but not RSK2, strongly activates both transiently-transfected and stably-integrated reporters. While RSK2 and MSK1 both phosphorylate H3-S10, only MSK1 can phosphorylate H3-S28 efficiently. Our data therefore suggest that phosphorylation of H3-S10 alone is not sufficient to activate transcription. Instead, phorphorylation of H3-S28 alone or in combination with phosphorylation of S10 is required for transcriptional activation. We further showed that targeting of MSK1 to endogenous genes also strongly activates their transcription. This not only induces expression of transcriptionally-poised genes, but also re-activates silenced genes. Successful recruitment of MSK1 to these genes is associated with high levels of phosphorylated H3-S10 and S28 at their promoters, further supporting an active role for H3 phosphorylation in activating gene expression.

NUCLEOPORIN PHOSPHORYLATION BY PLK1 CONTRIBUTES TO NPC DISASSEMBLY

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Higher eukaryotic cells undergo a so-called open mitosis, during which the nuclear envelope (NE) breaks down and the cytoplasm and the nucleoplasm are mixed. Nuclear envelope breakdown (NEBD) comprises a series of events, including nuclear pore complex (NPC) disassembly, nuclear lamina depolymerization and retraction of the NE into the endoplasmic reticulum (ER). Phosphorylation of NE components is hypothesized to trigger many of these events. However, the direct consequence of phosphorylation has only been described for a few NE components. In order to understand the molecular requirements of NEBD, we are using an in vitro assay to study the contribution of protein kinases and other factors in NE disassembly. A pivotal step in NEBD is NPC disassembly, which results in the loss of the NE permeability barrier. In our assay, NPC disassembly can be scored by both the dissociation of GFP-tagged nucleoporins from the nuclear rim and by the nuclear influx of large TRITC-labeled fluorescent dextrans. We observed that several protein kinases, including the mitotic kinase polo-like kinase 1 (PLK1), directly contribute to NPC disassembly. Depletion as well as inhibition of PLK1 resulted in a strong delay of NEBD, which could be rescued by addition of recombinant PLK1.

Many nucleoporins are hyperphosphorylated during mitosis. The peripheral GLFG-repeat nucleoporin Nup98 is a prime candidate to initiate the mitotic disruption of the NE. Nup98 contains several predicted Plk1 phosphorylation sites, some of which we confirmed to be phosphorylated during mitosis. Nuclei bearing phospho-deficient mutations of PLK1 sites in Nup98 underwent NEBD with a comparable delay as observed in Plk1-depleted cells in vitro, indicating that Nup98 phosphorylation by PLK1 contributes to NPC disassembly. Another nucleoporin that is hyperphosphorylated during mitosis is Nup53. Nup53 is a scaffold nucleoporin belonging to the Nup53-Nup93 subcomplex. Phosphomimetic mutations comprising predicted PLK1 sites of Nup53 resulted in NPC-targeting defects in vivo as well as in the loss of interaction with the transmembrane nucleoporin Ndc1 and Nup155 in vitro. Thus, Nup53 is another candidate nucleoporin that may rely on PLK1-mediated phosphorylation to dissociate from binding partners at the NPC during NEBD. Taken together, our data implicate that PLK1-mediated phosphorylation of nucleoporins at the onset of mitosis plays an important role in mediating mitotic NPC disassembly.

ESTABLISHING A TRACEABLE INDUCIBLE SYNTHETIC HETEROCHROMATIN SYSTEM IN FISSION YEAST

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Factories within the nucleus concentrate protein machineries, also called nuclear bodies, to efficiently perform essential activities such as transcription, replication, splicing, mRNA export and DNA repair. Some of these activities require access to the DNA, which is wrapped together with histones forming a structure called chromatin. Chromatin can adopt two main structural states: (i) transcriptionally active, open euchromatin or (ii) transcriptionally repressed, closed heterochromatin. In interphase cells, the organization of chromatin appears to be non-random and arranged into compartments, which correlate with nuclear activities. For example centromeres, which are essential heterochromatic structures, tend to cluster at the nuclear periphery or the nucleolus.

Using fission yeast as a model organism we want to address a key question in the field of nuclear organization: whether the proximity of distinctive chromatin types to particular nuclear regions is a cause or a consequence of their structure and transcriptional status. In order to achieve this, we use a traceable, inducible system to trigger alterations of the chromatin structure and follow its localization by live cell imaging technology.

This work surely will strengthen our knowledge of nuclear organization, especially regarding the link between the structural state of the chromatin and its relative positioning in the cell nucleus.

NUCLEAR SCALING IS MEDIATED BY IMPORTIN Α AND NTF2 IN *XENOPUS*

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The size of the nucleus varies among different cell types, species, and disease states. We sought to elucidate mechanisms of nuclear size regulation by studying nuclear scaling, the phenomenon that nuclear size often correlates with cell size. Two closely related frog species exemplify scaling: *Xenopus laevis* animals, cells, and eggs are larger than *X. tropicalis*. We recapitulated nuclear scaling in vitro with egg extracts. Using the same chromatin source, nuclear envelope surface area was on average 2.3-fold greater in *X. laevis* extract than *X. tropicalis* extract, while changing the amount of DNA 2-fold had a minimal effect. Mixing the two extracts in different proportions produced a graded effect on nuclear size, providing evidence for titratable cytoplasmic nuclear scaling factors. Since lamin B3 (LB3) is required for growth of these nuclei, we measured LB3 import and found it to be nearly 3-fold faster for nuclei assembled in *X. laevis* extract. Surprisingly, LB3 import did not correlate with its total amount in the two extracts, being 2-fold higher in *X. tropicalis*, thus leading us to investigate the upstream factors that regulate import. While the concentrations of most nuclear transport factors were similar in the two extracts, importin α and Ntf2 showed marked differences, with importin α levels being 3-fold higher in *X. laevis* and Ntf2 levels being 4-fold higher in *X. tropicalis*. Through depletion and addition experiments we demonstrate that these two proteins are specific mediators of nuclear size regulation, with importin α increasing overall import rates and Ntf2 reducing permeability of the nuclear pore complex. The combined capacities of these two factors to modulate lamin B3 import were sufficient to account for interspecies nuclear scaling. Furthermore, we find that importin α also contributes to nuclear size changes during early *Xenopus* embryogenesis. Thus, these nuclear transport mechanisms are physiological regulators of both interspecies and developmental nuclear scaling.

APPROACHING PNC FUNCTION THROUGH CHEMICAL BIOLOGICAL APPROACHES

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The perinucleolar compartment is a unique subnuclear structure that forms in cancer cells. The more cells having these structures in the primary tumors, the higher the chance patients develop metastasis. The PNC is enriched with newly transcribed pol III RNAs and RNA-binding proteins that are primarily implicated in pre-mRNA processing including PTB and CUGBP. Inhibition of polymerase III disassemble the PNC. Moreover DNA structure is essential for the structural integrity of the PNC, suggesting that PNC may play a role in pol III transcription and gene expression regulation. To understand the function of the PNC, we intent to identify the cellular processes that are important for PNC structure. Additional to cell biological approach, we have initiated chemical biology approach, in which we search for small molecules that disrupt PNC structure through novel mode of function other than pol III transcription or DNA structure disruptors. The identification of cellular pathways that are interfered by these molecules could reveal the function of the PNC and its relevance in malignancy. Initial screening of over 6400 compound library produced 45 hits belonging to several chemical classes not yet described for theirs anti-cancer properties. Here we report one of the compounds, 696, which disassembles PNCs and has anti-cancer properties. In vitro 696 reduces PNC prevalence in a dose dependent manner that is corresponding to growth inhibition of cancer cells. Within the effective concentration, 696 does not intercalate DNA in vitro, indicating that it is unlikely to affect DNA structure. In addition, at the PNC inhibition concentration, pol III transcription is not significantly affected. These findings suggest that 696 disrupts PNC through a novel mechanism. In vitro evaluation of its anti-tumor properties show that 696 effectively inhibit anchorage independent growth in soft agar assays. In addition, 696 significantly reduces invasive behavior of cells in a matri-gel invasion assay. Further studies are required to identify the molecular pathway that links 696 and PNC function. Also additional future screenings of DNA damaging anti-cancer drugs can help to discover that mechanism more in details.

CHROMATIN ON THE MEGABASE SCALE: THE FRACTAL GLOBULE ARCHITECTURE AND ITS PHYSICAL PROPERTIES

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The human genome folds into a structure that is dense enough to fit inside the nucleus, but compatible with cellular processes such as gene expression and the cell cycle. Understanding how the genome folds can elucidate the role of chromatin structure in enabling these crucial cellular processes. We recently developed Hi-C, a method that couples proximity ligation of genomic loci with massively parallel sequencing in order to enable genome-wide discovery of long-range chromatin contacts (Lieberman-Aiden & Van Berkum et al., Science, 2009).

Combining theoretical arguments and original Monte Carlo simulations we demonstrate that on the scale of several megabases, the experimentally obtained distribution of chromatin contacts is consistent with a fractal globule: a dense, highly organized, and knot-free structure. First proposed on theoretical grounds in 1988, the fractal globule has never before been observed, either in silico or experimentally.

We use our simulations to explore the physical properties of the fractal globule. We show that unentangled polymers (e.g. chromatin fibers) tend to form fractal globules by condensation or relaxation in a constrained volume, and show how easily the fractal globule can unravel in comparison to other condensed polymer conformations. These effects could play a role in regulating chromatin architecture during the cell cycle.

The fractal globule organizes the genome into discrete spatial sectors, which correspond to contiguous chromosomal subregions. We demonstrate the capacity of individual sectors to rapidly unfold and re-fold without disturbing the global chromatin conformation, a mechanism that could play a role in gene activation. Furthermore, we show that the presence of a fractal globule structure does not interfere with the ability of individual loci to diffuse across the globule over time.

A NOVEL DNA ZIP CODE TARGETS THE *INO1* PROMOTER TO THE NUCLEAR PORE COMPLEX TO PROMOTE H2A.Z INCORPORATION AND TRANSCRIPTIONAL MEMORY

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Many active yeast genes localize from the nucleoplasm to the nuclear periphery and interact with the nuclear pore complex (NPC). DNA “zip codes” in the promoters of these genes confers interaction with the NPC. Some of these genes exhibit epigenetic transcriptional memory: for several generations after being repressed, they remain at the nuclear periphery, primed for reactivation. Transcriptional memory requires the histone variant H2A.Z. We find that targeting of the active and the recently repressed *INO1* gene to the nuclear periphery is controlled by two distinct and independent mechanisms. Peripheral localization of active *INO1* and recently repressed *INO1* depends on different zip codes, involves different interactions with the NPC and has different outcomes. A Memory Recruitment Sequence (MRS) in the *INO1* promoter is necessary and sufficient to promote both peripheral targeting and H2A.Z incorporation. The MRS element and the NPC protein Nup100 play an essential and specific role in transcriptional memory. In cells lacking either the MRS or Nup100, *INO1* transcriptional memory is lost, leading to nucleoplasmic localization after repression and defective reactivation of the gene. Therefore, the interaction of recently repressed *INO1* with the NPC affects its chromatin state and future rate of activation.

THE NOVEL NMD FACTORS DHX34 AND NAG ARE REQUIRED FOR ZEBRAFISH DEVELOPMENT

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Nonsense-mediated mRNA decay (NMD) is a highly conserved surveillance mechanism present in all eukaryotes that prevents the synthesis of truncated proteins by selectively degrading mRNAs harboring premature termination codons (PTCs). Seven genes (smg-1-7) that are essential for NMD were originally identified in the nematode Caenorhabditis elegans and orthologs of these genes have been found in several species, including vertebrates.

We previously conducted a genome-wide RNAi screen that resulted in the identification of two novel NMD genes, that are essential for proper embryonic development, and as such represent a new class of essential NMD genes in C. elegans, which we termed smgl (for smg lethal). We showed that the encoded proteins are conserved throughout evolution and their human orthologs, DHX34 and NAG, are required for NMD also in human cells (1).

Here, we show that the morpholino-induced depletion of Zebrafish DHX34 and NAG proteins results in severe developmental defects and reduced embryonic viability. We also show that DHX34 and NAG are required for degradation of PTC-containing mRNAs in zebrafish embryos. The phenotypes observed in both DHX34 and NAG morphants are similar to defects in Upf1, Smg-5- or Smg-6 depleted embryos, suggesting that these factors affect the same pathway and confirming that zebrafish embryogenesis requires an active NMD pathway (2).

1. Longman,D., Plasterk,R.H., Johnstone,I.L. and Cáceres,J.F. (2007) Mechanistic insights and identification of two novel factors in the C. elegans NMD pathway. Genes Dev., 21,1075-1085.

2. Wittkopp,N., Huntzinger,E., Weiler,C., Saulière,J., Schmidt,S., Sonawane,M. and Izaurralde,E. (2009) Nonsense-mediated mRNA decay effectors are essential for zebrafish embryonic development and survival. Mol. Cell Biol. 29, 3517-3528.

PREMATURE AGING: INSIGHTS FROM A MOUSE MODEL REPRODUCING SPLICING MECHANISM OF *LMNA* GENE LEADING TO THE HUTCHINSON-GILFORD PROGERIA SYNDROME (HGPS)

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Four A-type lamins are produced by alternative splicing of the *LMNA* gene, Lamins A, C, AΔ10 and Progerin. Lamins A and C are major components of the nuclear lamina, a complex molecular interface located between the inner membrane of the nuclear envelope and chromatin. They are also distributed throughout the nucleoplasm and are involved in numerous functions, including DNA replication, transcription and chromatin organization. Progerin is hardly detectable in normal cells but a single nucleotide substitution, recurrent in most of the typical HGPS cases, promotes activation of a donor splice site in exon 11 of *LMNA* pre-mRNA, allowing a large increase of Progerin transcripts over the other lamin A isoforms. Our mechanistic studies indicate that HGPS mutation changes the accessibility of the exon 11 5' splice site allowing its modulation by U1 snRNP and a subset of Serine Arginine (SR)-rich proteins, namely SRp55, SF2/ASF and SRp40. To determine whether tissue specific distribution of these splicing factors influences LMNA aberrant splicing, we developed a knock-in HGPS mouse model in which murine *LMNA* gene was replaced by a mutated version of the gene harbouring the equivalent single-base substitution. Using this model, we demonstrate that the aberrant splicing leading to Progerin expression is conserved from mouse to human. Consistently, heterozygous mice lived longer than homozygous knock-in mice but less than wild type. Mutant mice demonstrated many features characteristic of HGPS in human. Furthermore, we show that different tissues expressed different levels of Progerin, highlighting the importance of alternative splicing in the development of the disease. Given that the cryptic splice site can perfectly be used in the absence of activating mutation, this model will allow us to gain novel insights into the splicing regulation occurring during physiological aging. Based on this knowledge, we describe the identification of novel molecules that prevent usage of the exonic 5' splice site allowing to overcome deleterious effect associated with Progerin. These molecules will be useful for the comprehension and hopefully the treatment of some of the features associated with pathological and physiological aging.

MITOTIC CHROMOSOME STRUCTURE: IRREGULAR FOLDING OF NUCLEOSOME FIBERS?

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Mitotic chromosomes are essential structures for the faithful transmission of duplicated genomic DNA into two daughter cells during cell division. A long strand of DNA is wrapped around the core histone and forms a nucleosome. The nucleosome has long been assumed to be folded into 30-nm chromatin fibers. However, it remains unclear how the nucleosome or 30-nm chromatin fiber is organized into mitotic chromosomes, although it is well known that condensins and topoisomerase IIα are implicated in this process.
When we observed frozen hydrated (vitrified) human mitotic cells using cryo-electron microscopy, which enables direct high-resolution imaging of the cellular structures in a close-to-native state, we did not find any higher order structures, or even 30-nm chromatin fibers, but just a uniform disordered texture of the chromosome (Proc. Natl. Acad. Sci. USA, 2008). To further investigate the structure of mitotic chromosome, we performed small angle x-ray scattering or SAXS, which can detect regular internal structures in non-crystalline materials in solution. Mitotic chromosomes purified from HeLa cells were exposed to the synchrotron radiation beam at SPring-8 in Japan. Again, the results were striking: no structural peaks larger than 11 nm were detected. Therefore, we propose that the nucleosome fibers exist in a highly disordered, interdigitated state like a "polymer melt" that undergoes dynamic movement. We also postulate that a similar state exists in active interphase nuclei, resulting in several advantages in the transcription and DNA replication processes (Current Opinion in Cell Biology, in press).

FUNCTIONAL CHARACTERIZATION OF LHR, A RAPIDLY EVOLVING HETEROCHROMATIN PROTEIN THAT PLAYS A MAJOR ROLE IN HYBRID INCOMPATIBILITY IN DROSOPHILA.

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Over evolutionary time genomes diverge by acquiring new mutations, some of which isolate species by erecting reproductive barriers. The study of such loci is important because it sheds light on how fundamental developmental processes diverge and result in new species. Hybrid incompatibility (HI) genes contribute to speciation by causing the sterility and inviability of interspecies offspring. We have identified the *Lhr* (*Lethal hybrid rescue*) gene as a major cause of hybrid lethality between *Drosophila melanogaster* and its sibling species *D. simulans*. Hybrid sons from this cross are normally inviable; however by mutating the *D. simulans Lhr* ortholog, viable adult hybrid sons are recovered. We have found that the LHR protein localizes to pericentric heterochromatin and interacts with Heterochromatin Protein 1 (HP1). In contrast to HP1 which is a highly conserved protein, population genetic studies have revealed that LHR is rapidly diverging under adaptive evolution. Interestingly, although *D. melanogaster* and *D. simulans* share a common ancestor only 3-5 Myrs ago, major differences have evolved in their heterochromatic sequence content and distribution. Could localization to fast evolving heterochromatin be driving LHR protein sequence divergence? We used immuno-FISH to cytologically map LHR in both species. Unlike HP1, which is a general heterochromatin factor, we found that LHR localization is restricted to specific sequences within pericentric heterochromatin. Both LHR orthologs partially colocalize with the G/C rich dodeca satellite, even though the chromosomal distribution of this satellite has altered significantly between the sibling species. Moreover, we find that *D. simulans* LHR localizes in a wild type pattern when expressed in *D. melanogaster*. This demonstrates that adaptive evolution has not altered the rules of heterochromatin localization between the species. These results suggest that LHR does not mislocalize in hybrids but instead may cause hybrid lethality by perturbing heterochromatin states. We are testing this by investigating the nuclear organization of dodeca or other heterochromatin sequences in lethal hybrids.

GLOBAL CHROMATIN ORGANIZATION IS ALTERED IN THE PRESENCE OF A NUCLEAR ENVELOPE TRANSMEMBRANE PROTEIN

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Majority of the genes at the nuclear periphery tend to be inactive and altering their positioning to the interior of nucleus results in their activation. Proteins of the nuclear envelope can recruit chromatin with specific epigenetic marks and can also recruit silencing factors that add new epigenetic modifications to chromatin sequestered at the periphery. The potential of the nuclear envelope (NE) in regulating gene expression is central to the favored hypotheses to explain the molecular mechanism underlying many NE diseases. To identify nuclear envelope transmembrane proteins (NETs) that could influence gene regulation, we are testing the effects of overexpression of NETs on global chromatin organization. We have tested principally novel NETs identified in a proteomics analysis of rodent liver nuclear envelopes for global affects on chromatin by over-expressing them in cell lines carrying different GFP-chromatin markers. One of the new NETs causes global condensation of chromatin, particularly accumulating at the nuclear periphery. This NET further accumulates centromeres at the nuclear rim. Detailed analysis by live cell microscopy and FACS shows that in majority of cells it neither alters the cell cycle pattern nor arrests cells in any particular stage; however cells appear to fall out of cell cycle. Kinetic analysis and microscopy demonstrated that its overexpression leads to regular cell death, which is directly related to the levels of the protein expression. We expect that this would be mediated by modification of chromatin that interacts with the nuclear envelope. We are currently analysing by microarray for changes in gene expression, which are altered in its presence. That 24 other NETs including the well-characterized emerin, LBR and LAP2 had no effect on chromatin condensation serves as an internal control for the specificity of this NET function. Thus it appears that couple of these novel NETs can have profound regulatory influence on chromatin architecture.

A FLUORESCENTLY TAGGED TFIIH MOUSE MODEL AS A BIOMARKER FOR TRANSCRIPTIONAL ACTIVITY

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The basal transcription/repair factor TFIIH is a 10 sub-unit complex essential for RNA polymerase II (RNAP2) transcription initiation and DNA repair. In both these processes TFIIH acts as a DNA helix opener (by the enzymatic activity of XPB and XPD helicases), required for promoter escape of RNAP2 in transcription initiation, and to set the stage for strand incision within DNA repair. We recently generated a knock-in mouse model that expresses endogenously a fluorescent version of XPB and we mapped the levels TFIIH (XPB is never found outside TFIIH), in the entire organism. Our study shows that TFIIH concentration varies amongst cell types, but are strictly regulated and kept constant in cells belonging to the same type. More interestingly, we demonstrated here that TFIIH concentration is linearly proportional to the speed of mRNA production and that both TFIIH levels and transcriptional activity correlate with the proliferation status of cells. Therefore, we propose that our mouse model can be used as a living tool/marker of transcriptional activity opening possibility for scientific and industrial applications.

FUNCTIONAL NUCLEAR ARCHITECTURE CHARACTERISTICS AND THE TOPOGRAPHY OF TRANSCRIPTION

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Evidence resulting from a multitude of approaches describes the cell nucleus as a dynamic landscape harboring chromatin and non-chromatin nuclear structures, including chromosome territories, chromatin domains and loops, genes, nuclear bodies, splicing speckles, as well as molecular machineries that orchestrate transcription, co-transcriptional splicing, DNA replication and repair. Our present view of nuclear architecture has been substantially reinforced by the combination of quantitative 4D (space and time) or, 3D imaging methodologies of living and fixed cells with molecular biological tools to visualize different nuclear structures in distinctly different colors. The resolution limit of light optical microscopy has thus far been the Gordian knot preventing the disentanglement of nuclear organization. Electron microscopy, in contrast, despite its superior resolution lacks the possibilities of visualizing differently labeled structures, which is instrumental to explore their nuclear topography by light optical imaging. Our goal is to explore the topography of global and gene specific transcription in different cell types by employing innovative 3D light microscopic approaches with superior resolution, namely, Structured Illumination Microscopy (3D SIM) and Spectrally Assigned Localization Microscopy (3D SALM). Both approaches overcome the Abbey/Raleigh limit of optical resolution. In this context, we are documenting the nuclear distribution of different modified states of RNA Polymerase II in relation to the sites of nascent RNA production, transcription factors and chromatin modifying enzymes. The post-translational modification status of the C-terminal domain (CTD) of RNA Polymerase II has been implicated as a regulatory mechanism for mediating the polymerase activity from initiation through elongation to termination. Our current work is aimed at the localization of RNA Polymerases II with different CTD modification patterns at nanometer resolution with respect to chromatin domains, the interchromatin compartment and the perichromatin region. We wish to attain a correlation of this profiling to the binding and assembly of trans-acting factors, such as chromatin-remodelers and histone modifying enzymes that influence not only gene expression, but also chromatin organization and structure.

THE SPLICING INHIBITOR SPLICEOSTATIN A INDUCES ACCUMULATION OF UNSPLICED TRANSCRIPTS IN NUCLEAR SPECKLES

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Spliceostatin A (SSA) was recently described as a small compound that binds the SF3b subunit of U2snRNP, inhibits splicing, and promotes export of unspliced RNAs to the cytoplasm where they are translated into aberrant protein products (Kaida et al 2007). Here we show that treatment of U2OS cells with 100 ng/ml SSA leads to a progressive disappearance of spliced endogenous transcripts and simultaneous accumulation of unspliced products. In parallel, nuclear speckles become progressively larger in cells exposed to SSA. FISH analysis revealed that several unspliced transcripts accumulate in the enlarged nuclear speckles. In contrast, unspliced transcripts derived from a β-globin transgene failed to accumulate in speckles and were detected predominantly in the cytoplasm. Based on these results we propose that nuclear speckles sequester unspliced transcripts in the nucleus preventing their export to the cytoplasm. However, a subset of unspliced RNAs can escape the speckles and reach the cytoplasm, where they might give rise to abnormal proteins.

COMPREHENSIVE PROTEOMIC ANALYSES OF HUMAN NUCLEAR ENVELOPE PROTEIN INTERACTING FACTORS

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Nuclear envelope (NE) is a cellular structure that consists of inner (INM) and outer nuclear membrane (ONM) facing nucleoplasm and cytoplasm, respectively. The ONM is directly linked to the endoplasmic reticulum (ER), while INM contains unique group of membrane proteins (INMPs) that specifically and efficiently target to the INM. INM associates with chromatin through INMPs and is involved in various kind of chromatin function. Although the heterochromatin organization and transcriptional repression is implicated in INM, the detailed mechanisms of INM on chromatin function have not been uncovered.
As the first step to elucidate the link between INM and chromatin function, we performed comprehensive identification of INMPs. Human 293 cell lines expressing FLAG-tagged INMPs and INMP interacting proteins (Lamin A, Lamin B1, Lamin B receptor, SUN2, Emerin, Lap2α, Lap2β, and BAF) were established. Using extracts containing INMPs, FLAG-tagged INMPs and INMP interacting proteins were immunoprecipitated using an anti-FLAG antibody, and the proteins in the immunoprecipitates were analyzed by semi-quantitative mass spectrometry. In addition to INMPs used as the bait for immunoprecipitation, a total of 488 proteins, including other INMPs, SYNE2, LEMD2, LEMD3, and TMEM43, was identified. Furthermore, 12 nuclear pore complex components, 37 chromatin related proteins, 40 ER proteins, 35 mitochondrial proteins, 22 tubulin related proteins and 25 uncharacterized proteins were included in the identified proteins. Among the 5 uncharacterized proteins we analyzed, 3 proteins showed NE localization as observed for known INMPs, and are temporally designated as NEA 1 (nuclear envelop associated protein 1), NEA2 and NEA3, respectively. Further analysis of NEA3 suggested that NEA3 is a new INMP that associates with Emerin and a nuclear pore complex component, NUP205. We want to discuss the reliability and possibility of this proteomic screening for INM proteins, and the function of the newly identified NEAs.

LIVE CELL IMAGING OF XIC HOMOLOGOUS PAIRING IN MOUSE ES CELLS

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The initiation of random X inactivation in mouse ES cells is accompanied by homologous pairing events at the X-inactivation centre (Xic) locus. Two regions of the Xic have been implicated, the *Xpr* locus which may participate in triggering X inactivation in an XX cell, and the *Tsix*/*Xite* locus which regulates the choice of X chromosome to be inactivated. In early differentiation, both X chromosomes express low levels of Xist and its antisense transcript Tsix. At the onset of X inactivation, this symmetric situation is rendered asymmetric, as one of the two X chromosomes monoallelically down-regulates Tsix and up-regulates Xist. Homologous pairing of the two Xic loci has been proposed to participate in this symmetry-breaking step. However the mechanism and dynamics of this process, as well as the precise outcome of Xic pairing, have remained unclear as so far all analyses have been performed on fixed cells. Here we have inserted a TetO array immediately downstream of the two *Tsix*/*Xite* loci in female ES cells by homologous recombination. Expression of the TetR-mCherry protein enables us to follow Xic pairing events in living ES cells during differentiation. In parallel, we also generated ES cell lines carrying autosomally integrated TetO loci. By calculating the MSD (Mean square displacement - an index of locus mobility), in these different ES cell lines, we demonstrate that the mobility of both Xic and autosomal loci increases upon differentiation, suggesting that this is a global phenomenon during early differentiation. In the case of the Xic, this mobility likely facilitates the random collision between two Xic loci – and initiates pairing events. Using this system, we demonstrate that pairing (≤2um) lasts for approximately 50 minutes and is accompanied by decreased mobility suggesting that the two loci are constrained or tethered during this time. We also demonstrate that the immediate outcome of this pairing event is frequently asymmetric expression of *Tsix* or *Xist*. Shortly afterwards, monoallelic Xist RNA coating of the future inactive X chromosome is observed. Our study represents the first example of live cell imaging of two homologous loci in ES cells during early differentiation, revealing the remarkable dynamics of the genome during this time window, and demonstrating for the first time the functional output of Xic pairing events at the level of *Tsix*/*Xite*.

DISTANT REGULATORY INTERACTIONS IN HUMAN MUSCLE DIFFERENTIATION

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There is increasing evidence of physical interactions between distant regions of chromosomes in a variety of biological systems. However, it is often unclear whether such interactions play a role in gene regulation and how the possibility of gene regulation by distant regulatory regions should affect the computational prediction of *cis*-regulatory modules (CRMs) of gene expression. To explore the importance of distal regulatory regions in human gene regulation, we examine potential distal CRMs in human muscle differentiation. ChIP-chip experiments show that myogenic transcription factors (TFs) bind to computationally predicted CRMs that are both proximal to and distal (more than 20 kb away) from the transcription start sites of genes differentially regulated during muscle differentiation. Using chromosome conformation capture (3C), we show that two of these bound CRMs, which were also shown to drive myogenic gene expression in reporter assays, physically interact with their distant upstream or downstream gene promoters (PDLIM3 and ACTA1) in a differentiation-specific manner. Circular chromosome conformation capture (4C) experiments followed by Illumina sequencing reveal genome-wide interactions of these CRMs during differentiation and suggest both intrachromosomal and interchromosomal interactions.

CANCER DIAGNOSTICS BASED ON INTERPHASE SPATIAL GENOME POSITIONING

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One of the fundamental properties of mammalian genomes is their non-random organization within the three-dimensional space of the cell nucleus. The nuclear position of many genes and genomic regions changes during physiological processes such as proliferation, differentiation and, importantly, disease. We are exploiting changes in gene positioning patterns to develop a novel diagnostic strategy for the detection of cancer. Using an established human mammary epithelial cell culture model of early breast cancer, we identified several genes that specifically reposition during tumorigenesis. We have extended these studies to human tissues and, using an unbiased screening approach, we have identified several genes whose nuclear positions are robustly altered in breast cancer, as compared to normal breast tissue. These genes, used either singularly or in combination, are able to detect cancer tissues with high accuracy. The changes in positioning patterns are not the consequence of global spatial genome reorganization in cancer cells since we find repositioning events to be gene specific. Nor are the changes in position a reflection of genomic instability. Moreover, the repositioning events are specific to cancer and do not generally occur in non-cancerous breast disease. Additionally, the spatial positions of genes are highly consistent between normal tissues from multiple individuals. These results establish spatial genome organization as a novel diagnostic strategy in cancer detection. The same principle can be applied to distinguish tumor types, predict treatment outcome and prognosis, and the method can be generalized to any disease.

DYNAMICS OF SINGLE MRNP NUCLEO-CYTOPLASMIC TRANSPORT THROUGH THE NUCLEAR PORE IN LIVING CELLS

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The flow of genetic information in eukaryotic cells occurs via nucleo-cytoplasmic translocation of mRNAs. Knowledge of in vivo mRNA export kinetics through nuclear pore complexes (NPCs) remains poor in comparison to protein transport. In this study, we established a mammalian system that allowed the real-time visualization and analysis of single mRNA-protein complexes (mRNPs) during export. Using a transcription-inducible system, large mRNPs were followed during nucleoplasmic travels and NPC translocation. The in vivo rates of mRNP transport and export were quantified and compared. Bulk mRNP average travel-times from transcription to the NPC occurred within a long-minute time-frame, with no pileup at the NPC. Export inhibition demonstrated that mRNA-NPC interactions were independent of ongoing export. mRNP export was rapid (~0.5 sec) and kinetically faster than nucleoplasmic diffusion. The nucleoplasmic transport dynamics of intron-containing and intronless mRNAs were similar, yet the presence of an intron did increase export efficiency. Our results provide visualization on the single mRNP level of the various steps in gene expression within the nuclear environment, the interchromatin tracks through which mRNPs move, and demonstrate the kinetics of mRNP-NPC interactions and translocation.

TFIIH: MOLECULAR ENGAGEMENTS IN PROLIFERATIVE *VERSUS* POST-MITOTIC CELLS

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The basal transcription/repair factor TFIIH is a 10 sub-unit complex essential for RNA polymerase II (RNAP2) transcription initiation and Nucleotide Excision Repair. In order to investigate diseases associated with mutated TFIIH a knock-in mouse model expressing a fluorescently tagged TFIIH subunit (XPB protein tagged with both fluorescent YFP and HA) has been generated and allowed analysing the molecular behaviour of TFIIH in the entire organism. It has been demonstrated that in highly differentiated post-mitotic cells, such as neurons, TFIIH is immobilized on the chromatin during transcription, while in proliferative cells, TFIIH freely diffuse. To disclose the molecular mechanism that explains TFIIH's different dynamic behaviour in different cells we addressed two questions (i) "what are the partners of TFIIH in post-mitotic versus proliferative cells?) and (ii) " where is TFIIH bound in these different cells".
To answer the first question we have developed a proteomic approach coupled to ChIP meant to analyze purified protein complexes associated with TFIIH. This approach allow us, through chemical cross-linking, to stabilize protein-protein interactions taking place on the native TFIIH complex bound to the chromatin, and in this way, to get access to the detection of transient and labile interacting factors in both cells types. Using state of the art confocal laser scanning microscopes, the absolute number of TFIIH molecules in neurons has been quantified. The estimated number is 3 times higher than the number of estimated promoters. In view of this result and the results previously published showing that 90% of these molecules are indeed stuck on the chromatin, we postulated that TFIIH could be bound elsewhere than in the promoter regions of genes. Analysis of TFIIH occupancy along the genome is currently under progress using a genome wide approach. Immunoprecipitated DNA from both neurons and cultured cells, are analyzed initially by qPCR on chosen sites, then for a high-throughput analysis to cover the entire genome (ChIP-Sequencing).

TRANSPOSABLE ELEMENTS ARE GENOMIC LANDMARKS FOR DNA HYPOMETHYLATION SPECIFIC TO EMBRYONIC STEM CELLS

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Embryonic stem cells (ESCs) have a distinctive epigenome, which includes their genome-wide DNA methylation modification status, as represented by the ESC-specific hypomethylation of tissue-dependent and differentially methylated regions (T-DMRs) of *Pou5f1* and *Nanog*. Here, we conducted a genome-wide investigation of sequence characteristics associated with T-DMRs that were differentially methylated between mouse ESCs and somatic cells, by focusing on transposable elements including short interspersed elements (SINEs), long interspersed elements (LINEs), and long terminal repeats (LTRs). We found that hypomethylated T-DMRs were predominantly present in SINE-rich/LINE-poor genomic loci. The characterization of sequence information revealed that the enriched SINEs were relatively CpG rich and belonged to specific subfamilies. The enrichment for SINEs spread over 300 kb in cis around genes and there existed SINE-rich genomic domains spread continuously over 1 Mb, which contained multiple hypomethylated T-DMRs. Furthermore, SINE-rich genomic domains, which occupy about 40% of the genome, harbour most of hypomethylated T-DMRs. In conclusion, our data reveal the chromosome-wide association between ESC-specific DNA hypomethylation and genomic sequence property, which provides insight into the regulation of high-order chromatin structure.

EPIGENETIC REGULATION OF A CGMP-DEPENDENT PROTEIN KINASE GENE AND ITS EFFECT ON ANT BEHAVIOR.

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Division of labor among nest-mates is the hallmark of the eusocial insects (ants, bees, wasps, termites) making them some of the most ecologically successful insects. Behavioral plasticity, possibly regulated at the epigenetic level, may allow ants to adapt to rapid changes in the surrounding environment. Little is known about the molecular pathways and genes that mediate these behavioral shifts. To understand how environment influences behavior, we have studied an ant, *Camponotus floridanus* with two distinct worker phenotypes: majors and minors, which differ both morphologically and behaviorally. We show that the levels of a gene encoding for a cGMP-dependent protein kinase (*PKG*) are associated with changes in the behavioral of these ants: outside workers express higher levels of *PKG* when compared to inside workers, both in brain and fat body. We have targeted fat body *PKG* gene expression with the use of RNA interference, resulting in the down-regulation of *PKG*. In future, we hope to demonstrate that down-regulation of *PKG* is sufficient to cause behavioral shifts in both majors and minors. Finally, we will report on the epigenetic regulation, via DNA methylation or other epigenetic mechanisms, that underlies changes in *PKG* transcription. We will also analyze how the PKG-dependent behavioral switch correlates with altered chromatin patterns at specific genes or at the genome-wide level. Defining the molecular mechanisms that underlie behavioral flexibility would help us understand how these traits evolve among populations and species under different ecological conditions.

CTCF IS CRITICAL FOR HIGHER ORDER FOLDING OF THE Β-GLOBIN DOMAIN

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Distant regulatory elements physically associate with target genes, and direct mapping of these looping interactions provides a means to identify elements and their respective targets. Looping interactions can be comprehensively assessed using 5C technology. 5C employs highly multiplexed ligation mediated amplification and deep-sequencing to detect up to millions of unique long-range interactions in parallel. A major advantage of 5C over other related high-throughput methods is that interactions are mapped at high resolution (several Kb) and without requiring prior knowledge of the protein complexes that mediate these associations.

We used 5C technology to study the three-dimensional folding of a 1 Mb chromosomal domain containing the human β-globin locus in cells that express the locus (K562) and cells that do not (GM06990). The 5C interaction maps reveal known long-range interactions between globin genes and their distant regulatory elements (e.g. the LCR and 3'HS1), specifically in globin expressing cells. Strikingly, in K562 cells all CTCF binding sites in the 700kb region surrounding the β-globin locus were involved in long-range looping interactions with each other and with active promoters. In contrast in GM06990 cells, in which this domain is largely transcriptionally inactive, very few long-range interactions were detected. These results suggest a major change in higher order folding of chromosomal domain upon activation of resident genes and point to a role for CTCF in this process. This was confirmed by 5C analysis of the domain in K562 cells in which CTCF was knocked down.

To gain further insight into higher order chromatin folding we built 3D models of this domain in its active and inactive state using a novel approach that combines dense 5C maps with the Integrated Modeling Platform. These models confirm long-range interactions between genes and distal elements and reveal higher order chromatin folding properties.

A MECHANISM LINKING WHOLE-CHROMOSOME ANEUPLOIDY TO DNA BREAKS.

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Cancer is associated with aneuploidy, both structural defects in chromosomes and abnormal numbers of intact chromosomes. The contribution of chromosome breaks to tumorigenesis is well accepted because of the possibility of generating cancer-causing mutations. However, the role of whole chromosome aneuploidy remains a subject of debate, principally because mechanisms by which whole chromosome aneuploidy would affect cancer pathogenesis are poorly understood. We have now evidence supporting one mechanism by which whole chromosome aneuploidy can lead to DNA breaks, via mis-segregation of chromosomes into structures commonly observed in cancer cells called micronuclei. Focusing only on micronuclei that originate from mitotic errors and contain a whole chromosome, we will present evidence for a mechanism whereby whole chromosome micronuclei develop breaks due to aberrant DNA replication and repair. We identify changes in the chromatin state of micronuclei as well as defects in the assembly of nuclear pore complexes and will discuss the implications of these findings for the mechanism of DNA breakage. Together, these results suggest a mechanistic link between errors in mitosis and the generation of DNA breaks.

PREVALENT LONG INTERGENIC NCRNAS (LINCRNA) ASSOCIATED PCG MEDIATED GENE SILENCING IN STEM CELLS

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Polycomb group proteins (PcG) mediate gene silencing by inducing a repressive chromatin state. Through trimethylation of histone H3 lysine 27 (H3K27me3), the polycomb repressive complex 2 (PRC2) establishes cell and lineage specific epigenetic landscape crucial for developmental process. Currently, it is not clear how these epigenetic modifiers like PcG are targeted to the chromatins. Recent studies indicated the involvement of non-coding RNA (ncRNA), particularly the long and transcribed from intergenic regions, as potential epigenetic regulators in the silencing mechanism to mediate or guide the interactions between PcG and chromatins. Coupling RNA immunoprecipitation (RIP) and RNA-sequencing (RNA-seq) technology, we attempt to identify and characterize a group of ncRNAs associated with PRC2 complex and H3K27me3 silencing in mouse embryonic stem cells by targeting Embryonic Ectoderm Development (Eed), a subunit of the PRC2 complex, followed by strand-specific RNA-seq approach. We performed deep-sequencing on biological replicates of Eed RIP-seq libraries and normalized against an input control. Among thousands regions found enriched for Eed binding, >70% of them were transcribed from non-coding and >30% were derived from intergenic regions; which overlapped significantly with previously identified large ncRNAs based on H3K4me3 and H3K36me3 maps. Anti-sense RNAs, particular in some interesting imprinting loci, can be found in portions of these regions. RNA polymerase II occupancy can be found at their 5' regions, suggesting these are potential *bona fide* transcripts. The delineation of their sequence contexts, their dynamics and diversities will provide fundamental knowledge on the mechanisms of how epigenetic regulation modulates transcription regulation and its impacts on the stem cell development.

CHROMATIN DYNAMICS OF COLLINEARITY

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Members of the *Hox* gene-family encode transcription factors that are required for proper patterning of mammalian body structures. A particular feature of *Hox*-genes is their presence in clusters, where their expression is regulated depending on their order on the chromosome templates, a process known as ‘collinearity’. Extensive *in-vivo* studies suggest that an intricate interplay between shifting epigenetically marked domains on the one hand, and dynamic long-range chromatin interactions on the other hand, could be among the mechanisms driving collinear transcriptional regulation. Currently though, little is known about elements involved in these regulatory mechanisms and how they influence each other.
Here, we present a high-resolution genome-wide 3C/seq assay (Chromosome Conformation Capture followed by high-throughput sequencing) that allows identification and quantitation of long-range chromatin interactions for multiple and closely spaced *Hox*-genes. A pilot experiment on differentially expressed members of the same *Hox*-clusters identifies gene-specific long-range chromatin interactions, confirming the high resolving powers of the assay. Current analyses of *Hox*-genes with differential transcriptional states, e.g. in tissues at different stages of collinearity and in mutant mice carrying modified *Hox*-clusters, is expected to generate insight into the role of chromatin dynamics in the process of collinearity.

INVESTIGATING THE STRUCTURAL AND REGULATORY LANDSCAPE OF THE *X-INACTIVATION CENTER* AT THE ONSET OF X INACTIVATION

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Early development of female mammals is accompanied by random inactivation of one of their two X-chromosomes. This process is controlled by the *X inactivation center* (*Xic*), a complex locus that produces the Xist non-coding RNA which accumulates on only one of the two X-chromosomes during differentiation and triggers its silencing. ES cells carrying large single-copy transgenes including *Xist* and up to 460kb of neighboring sequences are unable to activate the ectopic *Xist* locus - even though the endogenous *Xist* alleles can be up-regulated upon differentiation in the case of transgenic female ES cells. Therefore, critical long-range regulatory elements of *Xist* must exist. Identification of these loci is essential for our understanding of the mechanisms underlying the initiation of X-chromosome inactivation. Here we used Chromosome Conformation Capture Carbon-Copy (5C), a high-throughput 3C-based approach, to characterize the network of physical interactions at the *Xic* and are assessing the extent to which it integrates with the regulatory changes that take place at this locus, and *Xist* in particular, during the onset on X-chromosome inactivation.

A BIOCHEMICAL ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS INVOLVED IN TRANSPORT ACROSS THE NUCLEAR PORE COMPLEX

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Nucleocytoplasmic transport occurs through the nuclear pore complex (NPC), which in yeast is a highly symmetric ~50 MDa complex consisting of approximately 30 different proteins. Small molecules can freely exchange through the NPC, but macromolecules larger than ~40 kDa such as proteins, mRNAs, and ribosomal subunits must be aided across by shuttle proteins (karyopherins, or Kaps). Kap-mediated transport involves FG-nups, a family of NPC proteins. While much has been learned about the mechanism of nucleocytoplasmic transport, many details are still unknown; perhaps among the most important missing details is the binding kinetics of almost all the transport relevant interactions, due to significant technical challenges. The aim of this work is to analyze the protein-protein interactions involved in Kap-mediated transport across the NPC, using biochemical, biophysical, and cell biological approaches. Yeast karyopherins and full-length FG-nups are enriched from bacteria, and their affinities are studied quantitatively. These interactions exhibit a very tight apparent affinity – too tight to be compatible with the transport kinetics observed in vivo. The presence of competitor proteins and changes in bait protein distribution are seen to effect apparent affinity of these interactions. Trends observed in vitro for Kap/FG-nup interactions were consistent with ex vivo observations of interactions of transport factors with Xenopus oocyte NPCs and also with in vitro measurements of transport through a synthetic NPC-based filter. This work has suggested a role for factors such as non-specific competition in determining the kinetics and selectivity of transport.

POGZ MODULATES HP1 DISSOCIATION FROM MITOTIC CHROMOSOME ARMS FOR CORRECT ACTIVATION OF AURORA B KINASE IN HUMAN CELLS

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Heterochromatin protein 1 (HP1) plays a critical role in heterochromatin formation and mitotic progression through its interaction with various proteins. We have identified a unique HP1α-binding protein, POGZ, using an advanced proteomics approach. Typical HP1-binding proteins interact with HP1 through a PxVxL motif; however, POGZ was found to bind to HP1α through a zinc finger-like (HPZ) motif. This HPZ-mediated binding to HP1 competed with PxVxL proteins and destabilized the interaction between HP1 and chromatin. Depletion experiments in HeLa cells confirmed that the POGZ HP1-binding domain was essential for normal mitotic progression and for the dissociation of HP1 from mitotic chromosomes. Furthermore, POGZ was required for the correct activation and dissociation of Aurora B kinase from chromosome arms during M phase. These results reveal POGZ as an essential protein that links HP1 dissociation with Aurora B kinase activation during mitosis.

IDENTIFICATION OF A *CIS*-ELEMENT THAT REGULATES THE ASSOCIATION OF COHESIN WITH CHROMOSOMES.

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In eukaryotic cells Smc complexes (structural maintenance of chromosomes) mediate higher order chromosome structure for chromosome segregation, transcription and DNA repair by tethering together different strands of chromatin. How Smc complexes bind chromatin and how they tether DNA remains unclear in part because of the absence of any physiologically relevant in vitro assays. Here we report a novel in vitro assay for the binding of the Smc complex, cohesin, to DNA using yeast extracts and beads coupled to DNA from cohesin associated regions (CARs) identified in vivo by ChIP. Our in vitro assembled cohesin-DNA complexes faithful recapitulates in vivo assembled cohesin-DNA complexes including stability in the presence of 1.2M salt and dependence upon protein-DNA topology, cell cycle stage of the extract and ATP-dependent binding of Smc3p. This assay provides the foundation to elucidate the molecular basis of DNA binding and tethering activity of cohesin and by inference all Smc complexes. Our first analyses of cohesin binding to DNA suggest an intimate association between cohesin and DNA. Cohesin binds efficiently to naked DNA template but not to a chromatin template, and only 2-5 molecules of cohesin bind per 5 kb substrate. Furthermore, cohesin binds much more efficiently to DNA template with a CAR DNA than to a template without a CAR. We have mapped this sequence determinant to a 500 bp region in vitro and then shown that same sequence is important for cohesin binding to this CAR in vivo. Thus contrary to previous models cohesin is constrained to associate with specific DNA sequences. We are exploiting this assay to further characterize the properties of the cohesion reaction.

THE NSE3/MAGE AND NSE4/EID PROTEINS ARE INVOLVED IN TRANSCRIPTION REGULATION.

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Structural Maintenance of Chromosomes (SMC) complexes are involved in a wide range of cellular processes from cell division to gene regulation and DNA repair. In eukaryotes, three separate SMC protein complexes are conserved. Each contains a heterodimeric core of two SMC proteins interconnected by a kleisin subunit, together with from one to five more non-SMC elements (Nse). The complex containing SMC1 and SMC3, named cohesin, is responsible for sister chromatid cohesion during mitosis and meiosis. The condensin complex with SMC2 and SMC4 at its core is required for proper chromosome condensation and segregation during cell division. Both cohesin and condensin are also involved in gene regulation and DNA repair. The less well characterized SMC5/6 complex is involved in several DNA repair pathways.

The SMC5/6 complex is composed of two subcomlexes SMC5-SMC6-Nse2 and Nse1-Nse3-Nse4 (1, 2). Nse3 shows sequence similarity to the MAGE (Melanoma Antigen Gene) family of proteins, which are overexpressed in certain types of cancers and whose function has been linked to cell cycle regulation, apoptosis and neuronal development (3). Using site-directed mutagenesis, protein-protein interaction analyses and modelling of the Schizosaccharomyces pombe Nse3 onto the crystal structure of the MAGEA4 protein, we have identified surfaces on the C- and N-terminal domains of Nse3 that interact respectively with Nse4 and Nse1. The binding site for Nse4 is evolutionarily conserved within the Nse3/MAGE superfamily of proteins. Nse4 is related to the EID (E1A-like inhibitor of differentiation) family of transcriptional repressors (4). We show that there is a relatively promiscuous interaction between members of the MAGE family and those of the EID family with some specificity. MAGE proteins are able to relieve the transcriptional repression by EID proteins (see 5), again with some specificity. Our data suggest that the Nse3/MAGE and Nse4/EID proteins are involved in gene regulation.

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JADE PROTEIN FAMILY IN THE HISTONE ACETYL TRANSFERASE (HAT) HBO1 COMPLEX AND THE CELL CYCLE.

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Regulation of chromatin acetylation is important for chromatin remodeling and cell cycle. A small family of JADE proteins includes JADE1, JADE1L, JADE2 and JADE3, each bearing two mid-molecule tandem PHD zinc fingers. We previously demonstrated that JADE1 plays a role in maintenance of global histone H4 acetylation and enables HAT HBO1 to acetylate nucleosomal histones(1). Because HBO1 promotes DNA replication licensing and cell cycle progression we investigated a potential role of JADE proteins in the regulation of the cell cycle. Our results strongly suggest that similarly to HBO1, JADE1 is required for DNA replication and cell cycle progression. Moreover, manipulation of JADE1 expression altered expression and chromatin association of MCM and other replication factors. Chromatin association of JADE1 and HBO1 was also modulated during the cell cycle. Our data suggest that JADE proteins govern activities of HBO1 that are relevant to the DNA replication.

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MAMMALIAN SUN PROTEIN INTERACTION NETWORKS AT THE INNER NUCLEAR MEMBRANE AND THEIR ROLE IN LAMINOPATHY DISEASE PROCESSES

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The nuclear envelope (NE) LINC complex, in mammals comprised of SUN-domain and nesprin proteins, provides a direct connection between the nuclear lamina and the cytoskeleton, which contributes to nuclear positioning and cellular rigidity. SUN1 and SUN2 interact with lamin A, but lamin A is only required for NE localization of SUN2 and it remains unclear how SUN1 is anchored. We have identified emerin and short nesprin-2 isoforms as novel nucleoplasmic binding partners of SUN1/2. However, we demonstrated that tight association of SUN1 with the nuclear lamina depends upon a short motif within residues 209-228, a region that does not interact significantly with known SUN1 binding partners. Moreover, SUN1 localizes correctly in cells lacking emerin. Importantly then, the major determinant of SUN1 NE localization has yet to be identified. We further found that a subset of lamin A mutations, associated with laminopathies Emery-Dreifuss muscular dystrophy (EDMD) and Hutchinson-Gilford progeria syndrome (HGPS), disrupt lamin A interaction with SUN1 and SUN2. Despite this, NE localization of SUN1 and SUN2 is not impaired in cell lines from either class of patients. Intriguingly, SUN1 expression at the NE is instead enhanced in a significant proportion of HGPS but not EDMD cells and strongly correlates with pre-lamin A accumulation due to preferential interaction of SUN1 with pre-lamin A. We propose that these different perturbations in lamin A-SUN protein interactions may underlie the opposing effects of EDMD and HGPS mutations on nuclear and cellular mechanics. Mutagenesis studies are now underway to define the precise role of SUN1 interactions in determining its localization to the NE.

AN ACTIN-REGULATED IMPORTIN A/B-DEPENDENT EXTENDED BIPARTITE NLS DIRECTS NUCLEAR IMPORT OF MRTF-A

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Myocardin-related transcription factors (MRTFs) are actin-regulated transcriptional coactivators which bind G-actin through their N terminal RPEL domains. In response to signal-induced actin polymerisation and concomitant G-actin depletion, MRTFs accumulate in the nucleus and are recruited by their partner transcription factor SRF to promote transcription of target genes. Here, we study the mechanism by which MRTF-A enters the nucleus. We show that MRTF-A contains an unusually long bipartite nuclear localisation signal (NLS), comprising two basic elements separated by 30 residues, embedded within the RPEL domain. Using siRNA-mediated protein depletion in vivo, and peptide competition for nuclear import in vitro, we show that MRTF-A nuclear import utilises the classical nuclear import pathway. Both NLS basic elements are required for functional interaction with the Impα-Impβ heterodimer, which binds the RPEL domain competitively with G-actin. Thus, MRTF-A contains an actin-regulated nuclear import signal.

MECHANISM OF REPRESSION OF OLIGOMERIC ONCOGENIC TRANSCRIPTION FACTOR FUSIONS

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The role of oligomerization in chromosomal translocations leading to oncogenic transcription factor (TF) fusions in hematological malignancies has been elusive. It is known the multimerization domain of the fusion is required but how this contributes to tumorigenesis is poorly understood. These types of chimeric proteins often exert dominant-negative (DN) effects over their wild-type counterparts and de-regulation of downstream target genes. However, the mechanism of DN-activity has not been fully characterized. Here we show for acute lymphoblastic leukemia (ALL) associated fusions of PAX5 (over 20 distinct PAX5-fusion proteins reported) to C20orf112, DN-activity results from a very large increase in DNA-binding affinity due to multimerization of the PAX5 DNA-binding domain (DBD). This was observed by greater than an order of magnitude increase in the stability of chromatin binding in living cells as revealed by fluorescence recovery after photobleaching (FRAP). Mutational and biophysical analyses revealed that an ~35 aa C20 C-terminal α-helical region, which encodes a coiled-coil oligomerization domain, induces multimerization of the DBD, stable chromatin binding, and DN-activity. PAX5 DBD fusions to protein domains that induced di-, tri-, and tetramerization produced proteins with successively increasing stability of chromatin-binding and parallel increasing DN activities. Since mutations that inhibit PAX5-C20 multimerization, as well as over-expression of the C20 C-terminal domain, inhibit its DN-activity, small molecules that inhibit multimerization of this domain may be effective therapeutics for ALL in patients with PAX5-fusions to C20orf112. We extend these findings by discussing the high number of oligomerization domain fusions of PAX5, and other multimeric oncogenic TF-fusions, and how oligomerization of TF-fusions increases their affinity for chromatin thereby disrupting binding of wild-type counterparts to targets, thus generating sufficient DN-activity that contributes to oncogenesis.

WAPL AND COHESIN CONTROL HIGHER ORDER CHROMATIN STRUCTURE IN INTERPHASE CHROMOSOMES

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Mammalian genomes contain several billion base pairs of DNA, which is organized in a distinct set of chromosomes. DNA is wrapped around nucleosomes, and the resulting chromatin fibers are believed to be organized through helical folding or chromatin loop formation. How these higher order chromatin structures are generated is poorly understood, but recent observations indicate that the ring-shaped cohesin complex has a role in this process. Cohesin associates with specific sites in the genome throughout interphase and mediates cohesion between sister chromatids from S-phase until mitosis. In prophase, most cohesin dissociates from chromosomes in a process that depends on the Wapl protein, resulting in partial separation of chromosome arms. To understand the function of this prophase pathway we have generated mice in which the Wapl gene can be conditionally deleted. We show that Wapl is essential for proper chromosome segregation, cell division and viability. Remarkably, Wapl depletion also leads to stable accumulation of most if not all cohesin on chromatin in interphase and causes dramatic changes in chromatin compaction. In each interphase chromosome, cohesin accumulates in one axial domain which extends from one telomere to the other, similar to how the cohesin-related condensin complex forms a “scaffold” in mitotic chromosomes. We propose that cohesin binding sites in the genome form the base of chromatin loops, and that Wapl controls the number and dynamic properties of these loops by determining the occupancy of binding sites with cohesin.

CHROMATIN ORGANIZATION AND –DYNAMICS IN MOUSE STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS

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Stem cells are able to differentiate into different lineages depending on the elasticity of the substrate they grow on. It has been suggested that the gene expression changes during differentiation of stem cells into various cell types are partly regulated through alterations in higher order chromatin arrangements in cis and trans by structural proteins like non muscular myosin II (NMM II) and components of the nuclear lamina. This concept has gained support over the last few years by evidence that the positioning of genes within the nucleus can influence their expression. Our current studies aim at new insights into how proteins of the nuclear envelope and the cytoskeleton influence chromatin organization and mobility in mouse stem cells and mouse iPS cells. We employ different labeling approaches of chromatin and other nuclear structures of interest to observe chromatin organization and dynamics in living stem cells and at different time points during the reprogramming process of iPS cells.
Cells are transfected with constructs for H4 tagged with a photoactivatable GFP and H2B tagged with mRFP. This approach enables us to activate GFP fluorescence in selected areas and to track major chromatin movements with a spinning disk confocal microscope. To visualize changes in the localizaton of whole chromosome territories we use fluorescence tagged thymidine analogues, which are incorporated into newly synthesized DNA. Our results demonstrate an increase in nuclear deformation and major chromatin movements in stem cells as well as in fully reprogrammed iPS cells.
Using immunofluorescence in fixed cells we have detected differences in the expression and distribution of the SUN proteins that link the lamina to the cytoskeleton. Within the reprogramming proccess of iPS cells, the decrease of laminA expression was accompanied by a decrease of SUN2, while the expression of SUN1 remained unaffected or slightly increased.
To further examine whether these proteins influence chromatin organization and mobility, we have started to employ knockdowns for SUN1 and SUN2 as well as inhibitors of different components of the nucleo- and cytoskeleton.

WHAT SETS THE SIZE-LIMIT OF A NUCLEAR BODY? INSIGHTS FROM LOCALIZATION OF VARIOUS PORTIONS OF THE LONG NONCODING RNA, NEAT1, BY HIGH RESOLUTION *IN SITU* HYBRIDIZATION WITHIN PARASPECKLES.

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Current models of nuclear body formation by self-assembly through protein-protein interactions do not account for multiplicity of organelles of similar size within a nucleus rather than a single giant one. Recent studies implicated 2 long noncoding RNAs (lncRNAs), NEAT1/MENe/β, transcribed from the same promoter, as architectural elements of an ultrastructurally poorly-defined nuclear domain: Paraspeckles (PSP). Here, by immunoelectron microscopy we defined the ultrastructure of PSP and by high resolution in situ hybridization localized the short (3.7-kb) and the long (22.7-kb) NEAT1 ncRNAs to sub-regions within PSPs. Our results confirm identity at the ultrastructural level between PSP and the previously identified Interchromatin Granule-Associated Zones (IGAZ). We show that the 3.7-kb and the corresponding 5'-end of the 22.7-kb long NEAT1 transcripts are precisely localized at the periphery of the PSP/IGAZ, delineating strictly the frontier between the nucleoplasm and the nuclear bodies. Internal sequences of the 22.7-kb long ncRNA are specifically found in the interior of the PSP/IGAZ. Intriguingly, the 3'-extremity of the 22.7-kb NEAT1 overlaps its 5'-end and is also localised at the periphery of PSPs. This first localization of various portions of a single lncRNA transcript within the nucleus reveals its high level of compaction; suggests its looping out and implies a limit in size of the PSP/IGAZ. Consistently, measurement of PSP/IGAZ in HeLa cells set the limits of the diameter of these roundish or oblong nuclear bodies at 360 nm. To tentatively relate this threshold in size to the length of the transcript, we are now measuring PSP/IGAZ in mouse NIH3T3 cells in which the homologous transcript is shorter at 20.7-kb. Finally, conventional EM depicts a crown of relatively large fibers at the periphery and an electron-dense internal network that coincide with distribution of the two structural NEAT1 nc RNAs within the PSP/IGAZ. Underlining their different architectural roles, our results provide a rationale for transcription of two evolutionary-conserved ncRNAs from a single promoter.

NUCLEAR-RETAINED NON-CODING RNA REGULATES ALTERNATIVE SPLICING BY MODULATING SR SPLICING FACTOR PHOSPHORYLATION

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Alternative splicing (AS) of pre-mRNA is utilized by higher eukaryotes to achieve increased transcriptome and proteomic complexity. The serine/arginine (SR) splicing factors regulate tissue- or cell type-specific AS in a concentration and phosphorylation dependent manner. However, the mechanisms that modulate the cellular levels of active SR proteins remain to be elucidated. In the present study, we provide evidence for the role for the long nuclear-retained regulatory RNA (nrRNA), MALAT1 in AS regulation. MALAT1 localizes to nuclear speckles, interacts with SR proteins, and influences nuclear speckle distribution of splicing factors. Two independent sequence elements in MALAT1 are responsible for the localization of MALAT1 to nuclear speckles. MALAT1 is essential for cell viability and its depletion results in altered AS of several of the endogenous pre-mRNAs. Furthermore, MALAT1 regulates the cellular levels of phosphorylated form of SR proteins. Taken together, our results indicate that MALAT1 regulates AS by modulating the levels of active SR proteins. Our results further highlight a novel role for a long nrRNA in the regulation of gene expression.

HISTONE PRE-MRNA PROCESSING DEFECTS DISRUPT COILIN LOCALIZATION IN DROSOPHILA

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Cajal bodies (CBs) are conserved nuclear subdomains that contain high concentrations of the marker proteins coilin and SMN, as well as the spliceosomal small nuclear ribonucleoproteins (snRNPs) U1, U2, U4 and U5. They are thought to be sites for modification of snRNAs and snoRNAs. Histone locus bodies (HLBs) are related structures that that colocalize with the replication-dependent histone gene clusters. HLBs are thought to be critical for efficient histone mRNA formation in vivo. They contain factors required for histone gene transcription and pre-mRNA 3' end formation, including NPAT, FLASH and the U7 snRNP. HLBs and CBs are often found in close proximity to each other in human cancer cell lines and *Drosophila* nurse cells, but whether this represents a functional association is not known. Here we present a study of the interconnectivity of *Drosophila* CBs and HLBs by utilizing mutants in the snRNP and histone mRNA biogenesis pathways. Intriguingly, in a variety of both snRNP and histone biogenesis mutants coilin is delocalized, forming a kind of 'cloud' around the nurse cell HLBs. This phenotype was extremely penetrant. Notably, HLBs remained largely unaffected in the majority of these backgrounds. The strikingly similar phenotypes in each of these classes of mutation suggest that CB integrity is dependent on ongoing histone pre-mRNA biogenesis.

A CHROMATIN INSULATOR IMPOSES EARLY REPLICATION TIMING

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The nuclear genomes of vertebrates show a highly organized program of DNA replication where GC-rich isochores are replicated early in S phase, while AT-rich isochores are late replicating. GC-rich regions are gene dense and are enriched for active transcription, suggesting a connection between gene regulation and the molecular mechanisms responsible for a well ordered firing of DNA replication origins. Insulator elements can organize independent domains of gene transcription and are suitable candidates for being key regulators of replication timing. We have tested the impact of inserting a strong replication origin flanked by the β-globin HS4 insulator, on the replication timing of naturally late replicating regions. We find that the HS4 insulator has the capacity to impose early activation on a replication origin resulting in a dramatic shift in replication timing of the target locus from the second half of S-phase to the first half. Conversely, the presence of a proximal gene promoter that is highly acetylated and transcriptionally active does not convey any timing shift. We suggest that the high frequency of elements like HS4 along GC-rich isochores might contribute to their early replication.

GENOME-WIDE METHYLATION PROFILING FOR THE IDENTIFICATION OF NEW IMPRINTED GENES

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Imprinted gene expression depends on sex-specific methylation marks established in parental germ lines and maintained in the developing embryo after fertilization. The DNA-methyltransferase co-factor DNMT3L plays a key role in gametic methylation by enhancing the *de novo* methylating activity of DNMT3A on specific genomic sequences that are characterized by a combination of genomic and epigenetic features. Some 100 imprinted genes have been identified in mammals and abnormal expression dose of these genes is linked to growth and neurodevelopmental pathologies. Despite their key role in mammalian development, accurate estimation of the total number of imprinted genes is currently lacking, principally due to their stage- and tissue-specific nature of their mono-allelic expression.
We are aiming towards the unbiased identification of new imprinted control regions, through genome-wide methylation profiling of imprint-deficient mouse embryos derived from the *Dnmt3L* mutant model. *Dnmt3L-/-* females are viable but produce oocytes devoid of maternal methylation marks. From these imprint-free oocytes, we derived maternal imprint-free embryos by normal fertilization and total-imprint free embryos by parthenogenesis and used Methylated DNA Immunoprecipitation followed by high throughput sequencing to compare their methylation profile with stage-matched wild-type embryos (8.5dpc). As gametic imprinted methylation marks are transmitted to all somatic cells of the embryo, we would be potentially able to identify all the existing ICRs and the genes under their control by crossing our MeDIP-Seq data with transcriptome data that we obtained previously on expression microarrays. With this study, we have set up pioneering genome-wide experiments in the field of genomic imprinting where most current studies have focused on specific loci. We should be able in this way to provide major insight into an estimation of the real number of imprinted genes in the mouse genome and their impact on specific developmental pathways.

REGULATION OF MRNA EXPORT BY THE PHOSPHATIDYLINOSITOL 3 KINASE/AKT PATHWAY

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There are two multiprotein complexes involved in mRNA export. At the 5' end of the mRNA, the TREX/THO complex forms by sequential addition of UAP56, ALY/REF, and NXF1/TAP. NXF1 can then dock the complex at nuclear pores for export of the mRNA to the cytoplasm.
A second complex forms at the junction formed during splicing between two exons. This Exon Junction Complex (EJC) is built on a core of eIF4A3, Y14, MAGOH, and MLN51. Some mRNA export proteins, such as the essential mRNA export factor UAP56, have been reported at both locations on mRNA, so it may be that both complexes come together in the three dimensional structure of the mRNP complex.
A previous report identified an AKT phosphorylation site on ALY/REF. This same site is necessary for efficient mRNA export. In this study we show that the PI3K/AKT signal transduction pathway can regulate the binding affinity of the TREX/THOC associated proteins UAP56 and NXF1 in complexes formed at RNA splicing speckled domains and in the nucleoplasm. The core EJC proteins eIF4A3, MAGOH, and Y14 also have a binding affinity that is regulated by the PI3 kinase / AKT pathway. We also show that the active PI3 kinase/AKT pathway causes retention of polyA RNA in the nucleus, consistent with this signal transduction pathway regulating the export of bulk mRNA.
While the biochemistry of mRNA processing and export is increasingly well characterized, the regulation of these steps in mRNA metabolism is less understood. Live cell studies should play an important role in elucidating the mechanisms by which signal transduction pathways regulate mRNA export.

NETWORKING THE NUCLEUS: DOES FORM PRECEDE OR FOLLOW THE FUNCTION?

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Genes communicate with each other in space, and communication patterns rewire over time, making a specific topological organization of chromosomes that ensures efficient and coordinated expression of sets of genes. Decomposing nuclear architecture into well-defined networks—networking the nucleus—and studying the connections and communicability between networks provides a useful new framework for investigating complex organization in reprogramming the nucleus. We aim to test the hypothesis that nuclear reorganization predetermines cell-type specific transcriptional networks, using the lineage specifying factor MyoD as a model system to study the relationship between spatial nuclear architecture and gene expression in mouse and human muscle cells. Identifying the role of nuclear architecture in establishing the future transcriptional potential of a cell to execute a program of differentiation will provide insight into whether form precedes or follows function, and whether architecture is a critical element driving cell-type specific processes.

DROSOPHILA TOPOISOMERASE II ASSOCIATION WITH CHROMATIN IS DELIMITED BY INSULATORS AND DEMARCATES GENOME-WIDE TRANSCRIPTION AND REPLICATION.

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Insulators are DNA-protein complexes involved in higher-order chromatin organization, which may in turn, via this organization regulate gene expression. In *Drosophila* multiple insulators [(Su(Hw), dCTCF, CP190, Mod(mdg4)] exist that bind DNA at thousands of different and colocalized ChIP-chip identified sites. However, in an intact diploid nucleus these insulators all coalesce to 20-25 sites. Current models propose that insulators are involved in chromatin loop formation where multiple insulators from distinct loci within the genome coalesce via protein-protein interactions to form multi-complex entities termed “insulator bodies.” Insulator bodies form functional domains sealing the loops and prevent interference or crosstalk from regulatory regions on other loops or domains. Current evidence demonstrates that Topoisomerase II (Top2) proper function is essential for insulator function. Top2 is an evolutionary conserved nuclear protein that has been characterized as having both an enzymatic and nuclear structural function. It is involved in many critical cellular functions such as replication, transcription, recombination and genome stability. Genetic data show that loss of Top2 prohibits proper insulation. In addition, we demonstrate that proper Top2 function is necessary for formation and localization of Su(Hw) and Mod(mdg4) insulator bodies. The intention of this study was to further investigate the role of this protein in epigenetic regulation. Using ChIP-chip in *Drosophila* KC cells we were able to determine the localization of Top2 along the fly's genome. We show that the organization of Top2 along the genome does not have a direct binding correlation with the insulator proteins Su(Hw), Mod(mdg4) or dCTCF, but does show a statistically relevant novel association with the insulator protein CP190 and BEAF. It seems that CP190 and BEAF delimit the functional regions for Top2 binding. In addition Top2 binding correlates with Smc1 - a subunit of cohesin, origins of replication (ORI), PolII and gene expression. These findings provide a lead towards a possible mechanism for insulator function in the regulation of gene expression via insulator interactions with Top2. These finding may provide a defined role for Top2 in epigenetic regulation and will help to further enrich our current understanding of nuclear organization and gene regulation.

THE PML NB-DEPENDENT LOCALIZATION OF DAXX DURING MYOGENESIS

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Promyelocytic leukaemia nuclear bodies (PML NBs) are heterogeneous and dynamic nuclear structures where proteins involved in several cellular processes including apoptosis, DNA repair, and gene regulation can be sequestered. The PML NB localization of several of these proteins is required for their proper functioning and modification. One such protein is Daxx, whose involvement in chromatin remodeling, transcription repression, and apoptosis is dependent on its PML NB localization. The mechanism by which PML NBs coordinate all these functions remains unclear. Using the murine C2C12 myogenesis system, we show that the localization of Daxx during differentiation is PML NB dependent. In undifferentiated myoblasts, Daxx is localized to PML NBs. However, in fully differentiated multinucleated myotubes, the number of PML NBs decreases and Daxx is relocalized to chromatin, which is evident by its enrichment at pericentric heterochromatin domains known as chromocenters. The chromatin localization of Daxx and the otherwise normal decrease in PML NBs can be disrupted when the HDAC inhibitor TSA is added during differentiation. In the hyperacetylated state, a fraction of Daxx is sequestered to the PML NBs that remained during differentiation. Furthermore, when the fully differentiated myotubes were treated with interferon, PML NBs reformed and a fraction of Daxx localized to the reformed bodies. These findings demonstrate that the localization of Daxx during myogenesis is PML NB dependent and suggest a possible regulatory role for PML NBs during differentiation by controlling the subnuclear localization and thereby Daxx functions.

A FRACTION OF MCM 2-7 PROTEINS REMAINS ASSOCIATED WITH REPLICATION FOCI DURING A MAJOR PART OF S-PHASE

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The essential role of MCM 2-7 proteins in the initiation of DNA replication in all eukaryotes is well known. Their role in replication elongation is supported by numerous studies, but there is still a knowledge gap in this respect. Previous immunofluorescence studies in mammalian cells have shown that MCM 2-7 proteins are, after replication initiation, displaced from sites of DNA replication. Therefore, we used a robust statistic method to more precisely analyze immunofluorescence localization of MCM proteins with respect to DNA replication foci. We show that despite the predominantly different localization of MCM and replication signals, there is still a small, but significant fraction of MCM proteins that co-localize with DNA replication foci during most of S phase. The fluorescence localization of MCM proteins and DNA replication may thus reflect the fraction of the MCM complex functionally active at the replication fork, which supports a role of the MCM complex as the replicative DNA helicase.

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TRANSCRIPTION, RNA PROCESSING, AND NUCLEAR BODIES

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Of the many nuclear bodies that have been described, four are widely conserved phylogenetically among eukaryotic organisms: nucleoli, histone locus bodies (HLBs), Cajal bodies (CBs), and speckles (interchromatin granule clusters). Each has a known or probable role in transcription or RNA processing, but the relationship between the organelle and its molecular function(s) is different in each case. Nucleoli are the best understood. They occur in all cells that are transcribing rRNA, where they are physically associated with the rDNA loci on one or more chromosomes. In essence a nucleolus represents an active gene locus, along with its transcripts and multiple associated factors. Other active loci have a similar structure, such as the Balbiani rings of Chironomus and the loops of lampbrush chromosomes. The obligate association with rDNA is most clearly shown in the extrachromosomal nucleoli of amphibian oocytes, each of which contains a cluster of amplified rDNA genes. HLBs, as their name implies, occur regularly at histone gene loci. Instead of histone transcripts, they contain factors involved in processing these transcripts, such as the U7 snRNP. In the amphibian oocyte, HLBs exist independently of the chromosomes. Unlike the extrachromosomal nucleoli, they contain neither amplified histone genes nor their transcripts. The biogenesis of HLBs remains unclear: whether they arise at the histone locus itself or travel there after forming elsewhere is not known. CBs represent still a third class of nuclear structure. They contain a variety of factors involved in splicing, but are not regularly associated with specific gene loci. Because they do not contain nascent transcripts, they are unlikely sites of transcript processing. More probably, they are involved in the assembly and modification of the splicing machinery itself. CBs associate transiently with snRNA gene loci, but the significance of this association is not known. Finally, speckles or interchromatin granule clusters, represent a fourth kind of nuclear body. Like CBs, they contain splicing factors. Transient association between speckles and active transcription sites have been seen, suggesting that speckles may ferry splicing factors to nascent transcripts on the chromosomes. Comparative studies in a variety of cell types have been crucial in defining the structure and function of nuclear bodies.

DOWNREGULATION OF A HOST MICRORNA BY A VIRAL NONCODING RNA

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Herpesvirus saimiri -Herpesvirus family that infects T cells in New World primates, causing aggressive leukemias, lymphomas, and lymphosarcomas. HVS encodes seven Sm-class small nuclear RNAs called HSURs, forγ(HVS) is a member of an oncogenic *Herpesvirus saimiri* U RNAs, which are the most abundantly expressed transcripts in HSV-transformed monkey T cells. Besides binding Sm proteins, HSURs share other structural similarities with cellular Sm snRNPs, including a hypermethylated 5' cap and a terminal 3' stem loop. Nonetheless, HSURs exhibit no significant sequence similarity to any known cellular snRNA. HSURs are present in all HVS subgroups, with HSURs 1 and 2 being the most highly conserved and the only snRNAs expressed in the closely related *Herpesvirus ateles*. This high degree of conservation and prior genetic studies suggest an important role for HSURs in HVS-transformed T cells in vivo in infected monkeys. Bioinformatic searches revealed potential RNA-RNA interactions between certain miRNAs expressed in T cells and HSURs. Immunoprecipitation experiments confirmed the association of HSURs 1 and 2 with host cell miR-27, miR-16, and miR-142-3p in HVS-transformed cells, and mutational analyses demonstrated base-pairing interactions involving the seed sequences of miR-27 and miR-16. We found that one of the HSUR 1-bound microRNAs, miR-27, is targeted for destruction in transformed T cells. Transfection experiments showed that HSUR1 is both necessary and sufficient to down-regulate the level of miR-27. Moreover, the specificity of this regulation can be altered by mutating HSUR1's sequence to make it complementary to another microRNA. The destruction of miR-27 results in upregulation of host proteins that are targets of miR-27.
This is the first example of a virus producing a non-coding RNA to regulate host gene expression via the microRNA pathway. It establishes a function for HSUR 1, which can potentially be used for targeted destruction of specific microRNAs. We are currently studying the mechanism by which HSUR 1 targets miR-27 for decay, as well as the significance of the binding of miR-142-3p and miR-16 to HSURs 1 and 2 in virally transformed T cells.

LONG NON-CODING RNAS WITH ENHANCER-LIKE FUNCTIONS IN HUMAN

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Deciphering the function of the ever-growing class of long non-coding RNAs (ncRNAs) is one of the current challenges of biology. To assign a function to ncRNAs, we interrogated the transcripts annotated in the human genome, excluding protein-coding genes (PCGs) and their promoters. Using expression arrays, we detected 1,167 ncRNAs in multiple cell lines and observed an increased expression of a number of ncRNAs following differentiation of primary human keratinocytes. Unexpectedly, depletion of a number of ncRNAs in multiple cell lines revealed their positive regulation of their neighboring PCGs. Importantly, knock-down of an ncRNA adjacent to Snai1, a regulator of epithelial-mesenchymal transition, led to Snail1 depletion and concomitant defects in cellular migration.Expression of this ncRNA in an orientation-independent manner from a reporter construct driven by a thymidine kinase (TK) promoter demonstrated an RNA-dependent enhancer-like activity. These results reveal an unanticipated role for a class of enhancer-like RNA (elRNA) that acts to potentiate gene expression.

NUCLEAR DYNAMICS OF X-CHROMOSOME INACTIVATION

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In female mammals, one of the two X chromosomes is converted from the active euchromatic state into inactive heterochromatin during early embryonic development. This process, known as X-chromosome inactivation, results in the transcriptional silencing of over a thousand genes and ensures dosage compensation between the sexes. X inactivation is a dramatic example of mammalian epigenetics, involving differential regulation of two homologous chromosomes within the same nucleus, in a mitotically heritable but developmentally reversible manner. We are interested in the mechanisms and kinetics of this process in early mouse embryos and differentiating embryonic stem (ES) cells. Given the monoallelic character of X inactivation, we are particularly interested in the potential role of sub-nuclear compartmentalization in this process, both at the level of the master control locus of X inactivation, the Xic, and the non-coding Xist transcript it produces, that is responsible for inducing transcriptional silencing in cis. Our recent studies have shown that during female ES cell differentiation, the two Xics come into proximity of each other transiently just prior to the initiation of X inactivation and that this could participate in the mechanism through which an XX cell ensures the monoallelic up-regulation of Xist. We have also demonstrated that Xist RNA spatially segregates and reorganises the X chromosome in the nucleus during X inactivation, in collaboration with repetitive sequences, particularly LINEs. Our recent insights into these dynamic changes in nuclear organization of the X chromosome during ES cell differentiation will be presented.

PINNING DOWN NON-CODING RNAS IN CHROMOSOME REGULATION

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The role of non-coding RNAs in the structure and regulation of chromosomes will be discussed, including recent studies of XIST RNA, which induces silencing of one X chromosome in mammalian female cells. RNA from the X-linked XIST gene accumulates and paints the interphase chromosome territory of the inactive X chromosome (Xi), but how the RNA's binding, spread and localization is restricted to the parent chromosome is poorly understood, as is the mechanism(s) whereby some X-linked genes escape the silencing effects of XIST RNA. Recent findings regarding the factors involved in regulation of XIST RNA will be discussed, using an approach to manipulate chromosomal binding or release of XIST RNA directly within cells. Evidence suggests that XIST RNA's interaction with the chromosome and impact on its structure occur at the level of whole chromosome architecture, and that XIST RNA coats a repeat-rich inner core of the interphase chromosome which includes the condensed heterochromatic Barr Body. While it was previously thought that the Barr Body represents the silenced, heterochromatic protein coding genes of the Xi, evidence indicates that the Barr Body is comprised of silenced repetitive DNA and that many coding-genes position outside the condensed Barr Body. Further related studies investigating the potential role of interspersed repeats and repeat RNAs in the structure and regulation of chromosomes will be discussed. The implications for the role of “junk” DNA throughout the genome will be considered.

THE PERINUCLEOLAR COMPARTMENT ASSOCIATES WITH NOVEL RNA-PROTEIN INTERACTIONS

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The perinucleolar compartment is a nuclear body that forms in cancer cells and is highly enriched with a subset of small RNAs of pol III origin and RNA binding proteins primarily implicated in pre-mRNA processing. PNC enriching proteins and RNAs are localized to the PNC without their characterized interactive partners, suggesting novel RNA-protein interactions in the PNC. Here we provide evidence supporting this model. We found that RNA binding proteins, PTB and CUG-BP, when enriched in the PNC, behave differently from their nucleoplasmic counterparts with regards to their redistribution pattern in the absence of pre-mRNA synthesis and to their recovery dynamics after photobleaching. RNA pull down experiments show that MRP RNA co-precipitates both PTB and CUG-BP. Reciprocally, immunoprecipitations with specific anti-PTB or CUG-BP antibodies result in co-precipitation of MRP RNA. Evaluation of protein and RNA distribution using a high resolution fluorescence microscopic (OMX) system demonstrates that MRP RNA colocalizes with PTB and CUGBP in a reticulated network at the PNC. Furthermore, the network intertwines with newly synthesized RNA at the PNC. These data together demonstrate that MRP RNA is in a novel RNA-protein complex that nucleates at the PNC and may have a novel function in regulating gene expression of the PNC associated locus.

MULTIPLE STEPS LEADING TO THE ESTABLISHMENT OF SISTER CHROMATID COHESION

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Cohesin's stable association with chromosomes involves entrapment of chromatin fibres by its tripartite Smc1-Smc3-Scc1 ring via a poorly understood mechanism dependent on a separate Scc2/4 loading complex. A key issue concerns where entrapment initially takes place: at sites where cohesin is found stably associated or at distinct "loading" sites from which it translocates. Unlike functional cohesin, versions that cannot hydrolyse ATP co-localize with the Scc2/4 loading complex at core centromeres and at highly transcribed genes. In addition to Scc2/4, formation of these unstable "preloading" complexes requires Scc1's association with Smc1 and Smc3 nucleotide binding domains (NBDs), ATP-driven NBD engagement, cohesin's Scc3 subunit, and its hinge domain. We conclude that cohesin's association with chromosomes is driven by two key events. NBD engagement driven by ATP binding produces an unstable association with specific loading sites while subsequent ATP hydrolysis triggers DNA entrapment, which permits translocation for tens of kilobases along chromatin fibres. Establishment of cohesion during S phase, thought to involve co-entrapment of sister DNAs within a single ring, depends on acetylation of Smc3's nucleotide binding domain (NBD) by the Eco1 acetyl transferase. It is destroyed at the onset of anaphase due to opening of the ring as a consequence of Scc1 cleavage by separase. In yeast, Smc3 acetylation is reversed at anaphase by the Hos1 de-acetylase in response to Scc1 cleavage. Smc3 molecules that remain acetylated after mitosis due to Hos1 inactivation cannot generate cohesion during the subsequent S phase, implying that cohesion establishment depends on de novo acetylation during DNA replication. Lastly, by inducing Smc3 de-acetylation in postreplicative cells due to Hos1 over-expression, we show that Smc3 acetylation contributes to the maintenance of sister chromatid cohesion. A cycle of Smc3 NBD acetylation is therefore an essential aspect of the chromosome cycle in eukaryotic cells.

CONDENSIN AND MICROTUBULE TENSION DRIVE DE-CATENATION BY TOPOISOMERASE II THROUGH STABLE MODIFICATION OF DNA TOPOLOGY

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A longstanding question of mitosis is what drives Top2 action to remove all inter-chromosomal catenations between the replicated sister chromatids before cell division is completed. Here we show that catenated yeast minichromosomes undergo a topological transition during mitosis that provides a stable substrate for topo II to resolve inter plasmid catenanes in preference to intra plasmid supercoils. This transition requires a centromere, microtubule tension and Condensin function. Analysis of the supercoiling of plasmids purified from mitotic cells indicates that the transition is characterised by overwinding of the replicated DNA. We therefore describe the first experimental evidence that Top2 de-catenation during mitosis is driven by a topological modification of DNA mediated by condensin and spindle forces.

STRUCTURAL STUDIES OF THE BUDDING-YEAST KINETOCHORE

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The proteins of the budding yeast kinetochore associate into distinct subcomplexes, each with a specific role in building or organizing the complete assembly. The DNA-binding components include centromere-binding factor 3 (Cbf3, a heterohexamic complex of four protein species -- $Cep3p_2$:Skp1p:Ctf19p:$Ndc10p_2$), Cbf1 (a dimeric helix-loop-helix protein), Mif2p (a dimeric DNA-binding protein, the yeast homolog of CENP-C), and a nucleosome that contains a centromere-specific histone-3 homolog, Cse4p. We will report on crystal structures of some of these proteins, on electron microscopy of their interactions with each other and with DNA, and on biochemical experiments that integrate the structural data. We will relate these results to structural studies of "linker" components, such as the heterotetrameric Ndc80 complex.

THE EPIGENETIC BASIS OF CENTROMERE IDENTITY AND MAINTENANCE.

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Although the centromere is the basic element of chromosome inheritance, centromere function in mammals is specified not by DNA sequence, but by an undefined epigenetic mark. CENP-A, a histone variant that replaces H3 only at functional centromeres, confers a unique conformational rigidity to nucleosomes, providing the basis for such an epigenetic mark. The CENP-A centromere targeting domain (CATD) is sufficient to target histone H3 to centromeres and to generate the same conformational rigidity to the nucleosomes into which it assembles as does CENP-A. Depletion of CENP-A is lethal, but recruitment of normal levels of kinetochore proteins, centromere-generated mitotic checkpoint signaling, chromosome segregation and viability can be rescued by histone H3 carrying the CATD.

Epigenetic inheritance of centromere identity represents a problem for chromatin assembly, since replication of chromosomes and the redistribution of CENP-A nucleosomes to newly synthesized DNA strands requires that new CENP-A nucleosomes are assembled at the proper location following DNA replication. Prenucleosomal CENP-A is complexed with nucleophosmin-1, histone H4 and HJURP, the last of which is a chromatin chaperone and CENP-A chaperone/loader. Cell cycle-dependent recruitment of new CENP-A into nucleosomes at replicated centromeres is dependent on HJURP. Recognition by HJURP is mediated through the centromere targeting domain (CATD) of CENP-A, a region that induces a unique structurally rigidity to both the prenucleosomal CENP-A heterotetramer and the corresponding assembled nucleosome.

Through development and use of a covalent fluorescent pulse-chase labeling approach (SNAP tagging) CENP-A bound to a mature centromere is shown to be quantitatively and equally partitioned to sister centromeres generated during S phase, thereby remaining stably associated through multiple cell divisions. Loading of nascent CENP-A on the megabase domains of replicated centromere DNA requires passage through mitosis, but not microtubule attachment. Assembly and stabilization of new CENP-A-containing nucleosomes is restricted exclusively to the subsequent G1 phase, demonstrating direct coupling between progression through mitosis and assembly/maturation of the next generation of centromeric chromatin.

INTERPLAY OF KINETOCHORE GEOMETRY AND TENSION DETERMINES CHROMOSOME ORIENTATION DURING MITOSIS AND MEIOSIS

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Fission yeast centromeres, like metazoan centromeres, are composed of two domains, a kinetochore-assembling core centromere and heterochromatic peri-centromeric regions; a single kinetochore can attach several microtubules. By developing strategies to visualize the concealed cohesion within the centromere and to introduce artificial tethers that can influence kinetochore geometry, we show that cohesion at the core centromere induces a side-by-side configuration of kinetochores (mono-orientation) whereas cohesion at the peri-centromeric region promotes a back-to-back configuration (bi-orientation). However, the geometry of kinetochores is not the sole determinant of chromosome orientation, as artificial tethering of the core centromeres only partially induces mono-orientation during mitosis, in which homologous chromosomes are not connected. Moreover, sister kinetochores of achiasmate chromosomes (univalents), which are indeed mono-oriented in terms of kinetochore geometry, frequently attain merotelic (bipolar) attachment during meiosis I. Only when homologs are connected by chiasmata or even an artificial tether is this bipolar attachment efficiently converted to monopolar attachment. We propose a unified model for determining chromosome orientation during mitosis and meiosis in which the concerted action of kinetochore geometry and tension across chromosomes defines the stabilization of microtubule-kinetochore attachment, and thereby the final orientation of the chromosome.

HOW CENTROSOME DUPLICATION OCCURS EXACTLY ONCE PER CELL CYCLE

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The centrosome duplicates once per cell cycle in most dividing cells. The duplication process involves the alternating assembly and disengagement of centriole pairs at the core of each centrosome. Centrosome duplication has much in common with chromosome duplication, including a semi-conservative mechanism, a requirement for cyclin/Cdk2 activity, and the temporal separation of critical events occurring at the metaphase-anaphase transition and the G1-S transition. Based on studies with Xenopus egg extracts and cultured mammalian cells we showed that separase-dependent centriole disengagement at anaphase licenses centrosomes for duplication in the next cell cycle. Plk1 potentiates the disengagement process, in a manner similar to that for the separase-dependent destruction of cohesin. However, it is not known how disengagement in mitosis promotes new centriole formation in the subsequent cycle, or how new centriole formation is spatially controlled. The kinase Plk4 is required for centriole formation and is important for regulating centriole number: Plk4 overexpression causes centriole overduplication and can lead to de novo formation of centrioles, abrogating both temporal and spatial controls. We developed a system for studying Plk4-based centriole formation in Xenopus egg extract. We find that overexpression of the Xenopus Plk4 ortholog Plx4 causes overduplication of sperm centrioles as well as de novo centriole formation in the absence of input centrioles. Purification of epitope-tagged Plx4 from these extracts yields an interacting protein, Cep152, that colocalizes with Plk4 at the centrosome in mammalian cells, and is required for centriole duplication. We have also found that both Plk4 and Cep52 are highly upregulated during the differentiation of ciliated epithelial cells, which are unique in that they form hundreds of centrioles. The amplification of centrioles in these specialized cells occurs by a process that resembles in some respects the phenotype caused by Plk4 overexpression in cycling cells, suggesting that the mechanism of duplication is common but that the level of Plk4 pathway activation allows ciliated epithelial cells to overcome the once-and-only-once control.

USING MULTI-DIMENSIONAL PROTEOMICS TO DEFINE THE COMPLETE PROTEIN COMPOSITION OF MITOTIC CHROMOSOMES

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Mitotic chromosomes accomplish the critical segregation of the genome when cells divide, yet despite many decades of study remain poorly characterized with respect to their structure, mechanism of condensation and composition. In the present study, mass spectrometry identified >4000 polypeptides in prometaphase chromosomes isolated from chicken DT40 cells. Of these, >120 had been previously annotated as centromere proteins and 14 as telomere proteins. However, many of the proteins are likely to be cytoplasmic components that bind to the highly charged chromosomes after nuclear envelope breakdown. In order to identify novel proteins specifically associated with chromosomes, we used SILAC analysis to define four classifiers for each protein based on its level of enrichment in chromosomes relative to cytosol, its ability to exchange on and off of chromosomes in cytosol, and dependency on condensin or on Ska3 for chromosomal association. A fifth classifier was based on the estimation of relative copy numbers of each protein in chromosomes. Together, these classifiers can be used to distribute the >4000 chromosomal proteins in a multi-dimensional space. We then identified regions of this “SILAC space” enriched in proteins important for chromosome structure. As a validation of this approach, GFP tagging of 20 previously unknown proteins selected from this “space” revealed 10 to be novel centromere proteins, with another 8 to occupying other chromosomal domains during mitosis. One of the novel centromere proteins was found to occur in a complex with the previously described proteins Ska1 and Ska2. This novel protein, now known as Ska3, occupies a unique domain in the outer kinetochore, and was revealed by RNAi experiments to be essential for cell cycle progression in HeLa cells. The approach presented here offers a powerful way to define the functional proteome of complex organelles and structures whose composition is not simple or fixed.

EPIGENETIC GENOME CONTROL BY HETEROCHROMATIN AND RNAI MACHINERY

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Expression profiling of eukaryotic genomes has revealed widespread transcription outside the confines of protein-coding genes, leading to the production of antisense and non-coding RNAs. Studies in Schizosaccharomyces pombe and multicellular organisms suggest that transcription and non-coding RNAs provide a framework for the assembly of heterochromatin structures, which have been linked to various chromosomal processes. In addition to gene regulation, heterochromatin is critical for centromere function, cell fate determination as well as transcriptional and posttranscriptional silencing of repetitive DNA elements that are known to be major source of genomic instability. We have found that heterochromatin factors are widely distributed across euchromatic loci and collaborate with RNAi machinery to suppress antisense transcripts across large portions of the genome. Our recent progress in understanding the mechanisms of heterochromatin assembly, and the roles of RNAi and heterochromatin factors in epigenetic genome control will be discussed.

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MAKING CENS OF HETEROCHROMATIN

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Heterochromatin has several pivotal functions at centromeres. In fission yeast siRNAs, generated from outer repeat non-coding transcripts are incorporated into the RITS effector complex which is guided to homologous chromatin. This recruits the methyltransferase Clr4^{Suv39} which methylates nearby histone H3 on lysine 9 allowing binding of Swi6^{HP1} and other chromodomain proteins.

RNAi-directed heterochromatin: A key question is what mediates the recruitment of the Clr4 contain complex, CLRC, to transcript-bound RITS. We have identified a LIM domain protein, Stc1, that is required for centromeric heterochromatin integrity. Our analyses show that Stc1 is specifically required to establish H3K9 methylation via RNAi, and interacts both with the RNAi effector Ago1 (Argonaute, and with the chromatin-modifying CLRC complex. Moreover, tethering Stc1 to a euchromatic locus is sufficient to induce silencing and heterochromatin formation independently of RNAi. We conclude that Stc1 associates with RITS on centromeric transcripts and recruits CLRC, thereby coupling RNAi to chromatin modification.

CENP-A sub-kinetochore chromatin: Kinetochore assembly at a particular locus is directed by the deposition of the centromere specific histone H3 (Cnp1^{CENP-A}) variant in place of canonical H3. We previously demonstrated that an active RNAi pathway, the H3K9 methyltransferase and the Swi6^{HP1} are required to establish Cnp1^{CENP-A} chromatin over the central domain on freshly introduced centromeric DNA plasmids containing outer repeat and central domain DNA. Tethering of the Clr4^{Suv39} methyltransferase to a euchromatic locus results in the silencing of a nearby marker gene. The chromatin formed as a result of this Clr4^{Suv39} tethering has many features of native heterochromatin. Intriguingly, we find the establishment and maintenance of this form of heterochromatin is RNAi-independent. This indicates that the main role for RNAi in fission yeast is to recruit Clr4^{Suv39} activity and that tethering of Clr4^{Suv39} to a specific DNA binding site bypasses the need for RNAi. This synthetic heterochromatin can substitute for the centromeric outer repeats, the normal targets of RNAi at centromeres, and allow the assembly of sub-kinetochore Cnp1^{CENP-A} chromatin to form a fully functional centromere.

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ROLES OF RNA POLYMERASES IV AND V IN SIRNA-MEDIATED CHROMATIN MODIFICATION AND INTERPHASE CHROMATIN ORGANIZATION

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In Arabidopsis, pericentromeric repeats, retrotransposons and excess rRNA genes are assembled into heterochromatin and coalesce into higher-order nuclear structures known as chromocenters. The plant-specific nuclear DNA-dependent RNA polymerases, Pol IV and Pol V play important roles in heterochromatin formation by acting in a pathway in which 24-nt small interfering RNAs (siRNAs) mediate DNA methylation and repressive histone modifications typical of heterochromatin. Several proteins of this pathway, including RNA-DEPENDENT RNA POLYMERASE 2 (RDR2), DICER-LIKE 3(DCL3) and ARGONAUTE 4 (AGO4) colocalize with siRNAs within nucleolus-associated Cajal bodies. Key proteins of other siRNA or miRNA pathways also localize within the Cajal bodies. Collectively, these observations suggest that Cajal bodies are sites of assembly, storage or export of ribonucleoprotein complexes involved in small RNA metabolism in plants. At target loci subject to siRNA-directed DNA methylation, Pol V generates transcripts to which siRNA-AGO4 complexes bind, subsequently recruiting the de-novo DNA methylation and/or histone modifying machinery to the adjacent chromatin. However, Pol V also functions independently of the 24 nt siRNA biogenesis pathway in mediating the assembly of heterochromatin into chromocenters, coincident with the silencing of several classes of pericentromeric repeats. These results suggest that Pol V transcripts play a role in the formation of higher -order heterochromatic structures via at least two different mechanisms.

GENE PUNCTUATION: MULTIPLE ROLES OF TRANSCRIPTIONAL TERMINATION IN REGULATING EUKARYOTIC GENE EXPRESSION

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We are investigating the mechanism and regulation of transcriptional termination by RNA polymerase II (Pol II) in eukaryotes.

1) The basic mechanism of Pol II termination in all eukaryotes involves recognition of 3' end processing signals by dedicated factors followed by transcript cleavage either at the polyA site itself or at other co-transcriptional cleavage sites such as Rnt 1 sites in yeast (1) or CoTC sites in human (2). The exposed 3' product is then degraded by 5'-3' exonuclease which acts to destablise interaction between Pol II and the chromatin template so eliciting termination.
2) Transcriptional termination is closely linked to gene promoters through the formation of gene loop structures. The function of these gene loops is under investigation. Initial studies in S. cerevisiae show that gene loops across inducible genes are nuclear pore associated and may act to facilitate transcriptional memory (3). However a more general function may be to provide transcriptional fidelity to a gene. Thus loss of gene loop structure promotes bidirectional promoter activity resulting in the production of a new and abundant class of non coding RNA reading in divergent orientation to generate antisense transcripts.
3) Convergent genes (CG) in S. pombe employ a cell cycle regulated transcriptional termination process. In G1 CGs fail to terminate transcription which results in double strand (ds) RNA formation and consequent heterochromatin formation. This promotes CG cohesin recruitment in G2. Heterochromatin marks are then rapidly lost in G2 and Pol II transcription now terminates after each CG so that dsRNA is no longer generated (4). We now show that most genes encoding RNAi complexes are themselves convergent. Their consequent down-regulation in G1 when in a heterochromatin state allows transient release of centromeric heterochromatin in G1 by RNAi factor depletion. This in turn stimulates centromeric transcription which is required to maintain centromeric heterochromatin. In effect the convergent orientation of RNAi genes acts as an autoregulatory mechanism to maintain S. pombe heterochromatin.

Overall our results emphasize the surprisingly intricate network of gene regulation associated with Pol II transcriptional termination.

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CONSEQUENCES OF ANEUPLOIDY

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Aneuploidy is defined as a chromosome number that is not a multiple of the haploid complement. Almost a century ago, Theodor Boveri (1914) suggested a possible link between aneuploidy and cancer, a disease associated with high proliferative ability. On the other hand, aneuploidy at the organismal level is associated with severe developmental abnormalities and death in all species analyzed to date. These results raise an interesting conundrum. How is it possible that the presence of a single additional chromosome is associated with developmental defects characterized by growth restriction, yet in the context of cancer severe karyotypic abnormalities exist in cells with high proliferative abilities? We reasoned that in order to shed light on the relationship between aneuploidy and cancer it would be important to determine how aneuploidy affects the physiology and differentiation of normal cells. We generated 20 strains of budding yeast, with each strain bearing an extra copy of one or more of almost all of the yeast chromosomes (Torres et al., 2007). These strains display decreased fitness relative to wild type cells and share a set of traits: a cell proliferation defect (specifically a delay at the G1 – S phase transition), increased cell volume, increased need for energy, and increased sensitivity to compounds that interfere with protein folding and turnover. These shared traits, we find, are due to the additional proteins produced from the extra chromosomes. The analysis of mouse embryonic fibroblasts trisomic for either Chromosome 1, 13, 16 or 19 revealed similar shared traits (Williams et al., 2008). Trisomic MEFs exhibit a cell proliferation defect and signs of increased energy need and of proteotoxic stress. Based on these findings we propose that aneuploidy leads to a cellular response that is not unlike a stress response. In this "aneuploidy stress response" protein degradation and folding pathways attempt to correct protein stoichiometry imbalances caused by aneuploidy. This increases the load on these protein quality control pathways and results in proteotoxic stress, an increased need for energy and contributes to the cell proliferation defect shared by aneuploids. Most cancers are aneuploid and are thus likely to experience proteotoxic and energy stress. Identifying genetic alterations and compounds that enhance and suppress the aneuploidy stress response could therefore provide new insights into tumor evolution and provide new avenues for the treatment of a broad spectrum of tumors. Efforts aimed at identifying such will be presented.

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CONNECTING TRANSCRIPTION WITH MRNA PROCESSING AND CHROMATIN MODIFICATIONS

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The C-terminal domain (CTD) of the RNA polymerase II subunit Rpb1 undergoes dynamic phosphorylation, with different phosphorylation sites predominating at different stages of transcription. Our lab is studying how various mRNA processing and chromatin-modifying enzymes interact with the phosphorylated CTD to efficiently produce mRNAs. During early elongation, CTD serine 5 phosphorylation helps recruit the mRNA capping enzyme, the H3K4 methyltransferase COMPASS/Set1, and the Nrd1/Nab3/Sen1 early termination complex. Later in elongation, phosphorylation of CTD Serine 2 leads to recruitment of the H3K36 methyltransferase, polyadenylation factors, and the Rtt103/Rat1/Rai1 termination complex.

My lab's recent work is targeted towards uncovering further connections between these factors and analyzing their downstream effects. We have found that co-transcriptional H3K4 dimethylation and H3K36 methylation recruit the Set3C and Rpd3(S) histone deacetylases, respectively, to transcribed regions. These marks helps distinguish the downstream regions of genes from promoters, which have high levels of acetylation and high nucleosome turnover due to chromatin remodeling. We are studying how H3K4 and H3K36 methylations affect each other to establish the proper chromatin configuration for transcription. H3K4 methylation by Set1 also affects mRNA processing. Loss of Set1 affects termination by the Nrd1 early termination pathway, apparently by changing histone acetylation levels. In cells lacking H3K4 methylation, efficiency of intron splicing is also affected. Both of these effects may be mediated by changes in RNA polymerase II elongation rates.

Not only do modifications of the polymerase act to recruit the appropriate processing factors, it is becoming clear that RNA processing and packaging can in turn affect transcription. Failure to properly couple these processes may target defective RNAs for degradation. The connections between various steps in transcription and mRNA processing show that it is now necessary to think about gene expression as an integrated process.

CHROMATIN, POL II ELONGATION AND ALTERNATIVE SPLICING

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Regulation of alternative splicing (AS) through its coupling with transcription elongation can occur via changes in the RNA polymerase II (pol II) molecule itself or in chromatin structure. The first mode is illustrated by the effects of UV light on AS. The UV effect is not pleiotropic, is p53-independent, does not imply damage of the DNA template in cis, and is caused by inhibition of pol II elongation due to CDK-9-dependent hyperphosphorylation of its repetitive carboxy terminal domain (1).

The second mode of elongation control is illustrated by epigenetic modifications caused by siRNAs or external signals. When targeting promoter regions, siRNAs trigger transcriptional gene silencing (TGS), by promoting heterochromatin formation. We showed that siRNAs targeting intronic or exonic sequences close to an alternative exon regulate its splicing. The effect requires RNA:RNA hybridization with endogenous target transcripts, is AGO1-dependent and is counterbalanced by factors favoring chromatin opening or transcriptional elongation. The promotion of heterochromatin marks (H3K9me2 and H3K27me3) at the target site, the need for HP1α, and a reduction in pol II processivity suggest a mechanism involving the kinetic coupling of transcription and AS (2) This seems to be confirmed by genome wide ChIP-seq experiments indicating a differential convergence of AGO-1, but not AGO-2, DNA target sites and H3K9me2 marks between constitutive and alternative exons. Chromatin changes affecting AS can be also triggered by physiological signals. We found that membrane depolarization of neuronal cells promotes skipping of exon 18 from the neural cell adhesion molecule (NCAM). Whereas inclusion of this exon is increased by slow elongation, depolarization promotes its skipping by increasing elongation through the chromatin opening at NCAM via intragenic H3K9 hyperacetylation (3).

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HISTONE H3 LYSINE 9 TRI-METHYLATION AND HP1Γ FAVOR INCLUSION OF ALTERNATIVE EXONS

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Maturation of pre-mRNAs is initiated co-transcriptionally. It is therefore conceivable that chromatin-borne information participates in alternative splice decisions. Here, we find that alternative exons of the CD44 gene are made distinctive from the neighboring constant exons by tri-methylation of histone H3 on lysine 9 (H3K9me3). Upon activation of protein kinase C, this histone modification becomes coincident with HP1γ, a chromodomain protein specifically binding H3K9me3. This protein facilitates inclusion of alternative CD44 exons via a mechanism involving decreased RNA polymerase II elongation rate and increased spliceosome recruitment. In addition, its presence inside the gene correlates with increased contact between the chromatin and the CD44 pre-mRNA. Altogether, our data provides clear evidence of the localized impact of histone modifications on regulation of alternative splicing, and define HP1γ as possible bridging molecules between the chromatin and the splicing machinery.

CONNECTING THE GENOME TO THE CYTOPLASM – THE VIEW FROM GENETIC SCREENS TO THE SINGLE CELL

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We are interested in the regulated movement of proteins and RNAs between the nucleus and the cytoplasm. Some proteins shuttle between the nucleus and the cytoplasm in a highly regulated manner that depends on the cell environment. In particular, we have used the shuttling of a key transcription factor as a basis for a whole genome siRNA screen for factors that affect nuclear export at both the general and specific level in mammalian cells. In a second whole mammalian cell genome siRNA screen, we have identified genes that play critical roles in mRNA processing and localization. Together, we have generated networks of interactions that predict potential novel therapeutic approaches in cancer and neurological diseases. Lastly, we have developed strategies for determining the absolute number of mRNA isoforms in single cells. In doing so, we have been able to observe for the first time the relative variation in alternative mRNA processing and the implications for how cells respond to drugs and environment.

GENETIC ANALYSIS OF NUCLEAR BODY FUNCTION.

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The eukaryotic nucleus is a congested place and macromolecular crowding is thought to play an important role in increasing the relative concentrations of nuclear proteins, thereby accelerating the rates of biochemical reactions. Cajal bodies (CBs) are conserved nuclear subdomains that contain high concentrations of RNP assembly and processing factors, including the Survival Motor Neuron (SMN) complex and the uridine-rich spliceosomal small nuclear ribonucleoproteins (snRNPs). Similarly, Histone Locus Bodies (HLBs) accumulate factors required for histone gene transcription and pre-mRNA 3' end formation, including the U7 snRNP. Genetic ablation of key CB and HLB components shows that dispersal of these subdomains is not cell lethal, however, loss of these factors can result in a wide range of organismal phenotypes, from mild to severe. We will discuss recent progress towards understanding how nuclear body components come together, what happens when they form, and what benefit these subcellular structures provide to the organisms in which they are found.

FOLLOWING SINGLE MRNAS FROM SYNTHESIS TO DECAY IN LIVING CELLS

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Live cell imaging have been instrumental in analyzing the dynamic properties of gene transcription. New technologies in optical microscopy and fluorescent probe development have been pushing the envelope of our analysis capabilities. We have been dedicated to developing and implementing these technologies to further the understanding of transcription dynamics in cells and organisms. We have utilized computational approaches to analyze real-time transcription activities of both gene arrays and endogenous genes from yeast to human cells. We have employed a plethora of imaging methods, ranging from confocal and multiphoton microscopy, long-term cell imaging, fluorescence fluctuation spectroscopy, high-speed real-time widefield microscopy, single molecule tracking, and super-resolution microscopy. We have investigated key processes from initiation, elongation, termination, splicing, nuclear pore export, cytoplasmic trafficking, localization and decay. Rigorous mathematical modeling allowed us to extract quantitative kinetic parameters that accurately describe these processes in living cells.

MRNP REARRANGEMENTS DURING THE PIONEER ROUND OF TRANSLATION, NONSENSE-MEDIATED MRNA DECAY, AND THEREAFTER

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In mammalian cells, two different messenger ribonucleoproteins (mRNPs) serve as templates for protein synthesis. Newly synthesized CBP80/CBP20-bound mRNPs initially undergo a pioneer round of translation. One purpose of this round of translation is to ensure the quality of gene expression, as exemplified by nonsense-mediated mRNA decay (NMD). NMD largely functions to eliminate mRNAs that prematurely terminate translation, although NMD also contributes to proper gene control, and it targets CBP80/CBP20-bound mRNPs (for recent publications, see Sato et al., 2008; Isken et al., 2008). CBP80/CBP20-bound mRNPs are remodeled to eIF4E-bound mRNPs as a consequence of the pioneer round of translation as well as independently of translation (Sato and Maquat, 2009). eIF4E-bound mRNPs support the bulk of cellular protein synthesis and are the primary targets of mRNA decay mechanisms that conditionally regulate gene expression. Mechanistic aspects of NMD will be discussed, including how CBP80, which is acquired by the 5′ caps of transcripts within nuclei, promotes NMD at multiple steps by promoting mRNP rearrangements.

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HUMAN ISWI CHROMATIN REMODELING COMPLEXES IDENTIFY THEIR NUCLEOSOME SUBSTRATES VIA A CONTINUOUS SAMPLING MECHANISM

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Chromatin remodeling complexes are able to translocate nucleosomes along the DNA in an ATP-dependent manner. We have studied autofluorescent protein constructs of the human ISWI family ATPases Snf2H and Snf2L, the catalytically inactive Snf2L+13 splicing variant and the accessory Acf1 subunit in living human and mouse cells. By using a combination of advanced fluorescence microscopy bleaching and (cross-) correlation spectroscopy techniques the mobility, protein-protein and chromatin interactions were investigated. All proteins displayed average residence times of 10 - 400 ms in the chromatin bound state, and Snf2H and Acf1 formed a heterotetramer or higher order complex. The measured remodeler dynamics were indistinguishable for active and inactive Snf2H or Snf2L. The two ATPases were present at endogenous concentrations of 0.14 - 0.83 µM. We calculate that they continuously sample all nucleosomes within minutes in transient binding reactions to read out their propensity to be translocated. Only very few of these binding events lead to repositioning. Thus, most nucleosomes are stably tethered to their binding sites. Our quantitative analysis predicts that every nucleosome can be targeted efficiently to a new position if an appropriate signal is present. It defines the cellular response times for nucleosome repositioning by ISWI-type remodelers to be in the range of 0.3 - 10 minutes for their function as molecular switches that regulate DNA access.

SWI1, A COMPONENT OF THE FORK PROTECTION COMPLEX, PROTECTS REPLISOME COMPONENTS AGAINST DEGRADATION

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To ensure proper duplication and stability of the genome, DNA replication has to be faithful and is highly controlled. Arrested forks are among the most serious threats to genomic integrity. Indeed, they can collapse, break, or rearrange, and lead to activation of oncogenes or de-activation of tumor suppressor genes. We recently found, in Schizossacharomyces pombe, that the fork protection complex (FPC), Swi1-Swi3, plays a central role in checkpoint activation and replisome stabilization. Functions of FPC are conserved throughout evolution, and Swi1-Swi3 homologs are also involved in replication fork progression and stabilization. However, it is not understood how this complex actually acts at the replication fork. In this work, we investigate the effect of swi1 deletion on the behavior of replisome components. We show that replication fork progression is altered in the absence of Swi1. Indeed, we demonstrate that, in the absence of Swi1, replisome components, such as polymerases and MCM helicases, have a shorter half-life. It appears that Swi1 functions to stabilize replication fork components. Additionally, we show that the mts3-1 proteasome mutation stabilizes these replisome proteins. Taking together, this data suggest that Swi1, as a subunit of FPC, stabilizes the replication fork by protecting replisome components against proteasome-dependent degradation.

OPPOSING EFFECTS OF NUCLEOLAR ONCOPROTEINS ON RRNA TRANSCRIPTION

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Ribosomal RNA (rRNA) transcription is finely controlled by specific activator and repressor complexes that facilitate/prevent the assembly of the RNA polymerase I (Pol I) transcription machinery on the rDNA promoter regions. A few oncoproteins have been described to act either as activators or repressors of rDNA transcription. Here we show that two oncoproteins, MYC and MTG16, exert opposing effects on rDNA transcription. While MYC acts as an rDNA activator, MTG16a acts as an rDNA repressor, which is capable of counteracting MYC-induced rDNA transcription. The disruption of the delicate balance between these two oncoproteins, by either overexpressing MYC, or knocking down MTG16, in the same breast epithelial cell context, results in an increase of both rRNA synthesis and argirophilic nucleolar organizing regions (AgNORs) concomitant with altered breast epithelial acinar morphogenesis, recapitulating changes typical of early breast tumorigenesis.

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THE SUN DOMAIN CONTRIBUTES TO NUCLEAR ENVELOPE TARGETING OF HUMAN SUN2

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Attachment of the nucleus to the cytoskeleton is essential for processes like nuclear anchorage and migration. Nuclear-cytoskeletal connections are established by LINC (linker of nucleoskeleton and cytoskeleton)-complexes, which bridge the nuclear envelope (NE) and connect the nuclear lamina and the chromatin to the cytoskeleton. LINC-complexes are composed of SUN (Sad1p/UNC-84) domain proteins in the inner nuclear membrane (INM) and KASH (Klarsicht/ANC-1/Syne homology) domain proteins in the outer nuclear membrane (ONM). SUN and KASH domains are exposed to the perinuclear space, where they interact with each other. Targeting of SUN proteins to the INM is generally accomplished by their N-terminal nucleoplasmic domains, whereas KASH proteins depend on their luminal interactions with SUN proteins for ONM recruitment.

We studied INM targeting of human SUN2. Surprisingly, we found that in addition to N-terminal elements, the SUN domain contributed to NE localization of SUN2. Deletion or mutation of the SUN domain caused partial mislocalization of SUN2 from the NE to the ER and increased its mobility in the NE indicating that NE retention was affected. Consistently, fusion of the SUN domain of SUN2 to the ER-resident SUN family member SPAG4 (sperm associated antigen 4) was sufficient to target the chimeric construct to the NE. Furthermore, our results suggest that SUN domain-mediated NE localization relies on SUN-KASH interactions. First, overexpression of KASH proteins abolished NE targeting of SPAG4-SUN2 chimera. Second, we identified a SUN domain point mutant of SUN2 deficient in KASH binding, which showed impaired NE localization.

Our results show that the luminal domain of an INM protein can contribute to NE targeting. Further, they suggest that localization of SUN and KASH proteins is interdependent.

NOVEL CONSTITUENTS OF THE OUTER NUCLEAR MEMBRANE

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The outer nuclear membrane (ONM) represents a functional interface between the nucleus and the cytoplasm. This is accomplished in part through a class of membrane proteins containing C-terminal KASH-domains that translumenally associate with inner nuclear membrane SUN-domain proteins to facilitate their retention on the ONM. Throughout eukaryotic organisms this system of KASH- and SUN-domain proteins, called the LINC-complex, is utilized to influence nuclear positioning and movement of intranuclear structures. We have identified two novel mammalian KASH-domain proteins whose expression appears restricted to specific cell types. Dalek6 is found in germ cells during meiotic prophase I where it colocalizes with Sun1 and telomeres. We have demonstrated a functional association between Dalek6 and the dynein motor complex. It is our hypothesis that these interactions are required to move meiotic chromosomes to facilitate proper homolog synapsis. Another KASH-domain family member we have identified is lymphocyte-restricted membrane protein (LRMP). Predominantly expressed in developing lymphocytes, LRMP associates with the ER-resident calcium channel inositol 1,4,5-triphosphate receptor (IP3R). Our results indicate that this interaction is sufficient to recruit endogenous IP3R to the nuclear envelope and appears to modulate IP3R activity. Thus we propose that the LINC-complex may also serve to modulate calcium signaling critical to lymphocyte development. Together these proteins represent novel roles for the LINC-complex in mammalian cell biology.

THE BLOOM'S SYNDROME PROTEIN IS ESSENTIAL FOR THE CENTROMERIC DISJUNCTION PROCESS

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Bloom's syndrome (BS) displays one of the strongest known correlations between chromosomal instability and a high risk of cancer at an early age. BS is caused by mutations in the BLM gene which encodes BLM, a RecQ 3'-5' DNA helicase. Extensively studied as a key element in DNA metabolism in S-phase, BLM has now an emerging role during mitosis. Indeed, the BS phenotype includes a significant increase in the frequency of anaphase bridges and lagging chromosomes during cell division, suggesting a defect in sister chromatid separation during mitosis. Moreover, BLM localizes to anaphase bridges and to DAPI-negative ultrafine DNA bridges (UFBs) and is required for their resolution and possibly for their prevention. This suggests that BLM could be involved in the resolution of complex DNA structures persisting after completion of the DNA replication process. However, no data on BLM localization and function between S phase and late mitosis were available. We found that BLM localizes to centromeres from G2 to mitosis. BLM defect leads to structural and functional centromeric alterations that are associated with sister chromatid separation defect, thereby causing the chromosomal missegregations characteristic of BS cells. We also report functional interaction between BLM and factors involved in both centromere structure processing and centromere disjunction. These new function of BLM at centromeres is discussed in light of its significance in the physiopathology of the Bloom syndrome.

INHERITANCE OF HISTONE ACETYLATION AND H3K9 METHYLATION THROUGH S-PHASE IS REGULATED BY ATP-DEPENDENT CHROMATIN REMODELLER SMARCAD1

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During S-phase both genetic and epigenetic information must be faithfully replicated to guarantee the genomic integrity of both daughter cells. Here we identify SMARCAD1 (SWI/SNF related, Matrix associated, Actin dependent Regulator of Chromatin containing DEAD/H box), an ATP-dependent chromatin remodelling protein as a new regulator of epigenetic information, performing a key role in maintaining correct patterns of histone modifiction through S-phase.

Loss of SMARCAD1 results in increased histone acetylation, predominantly H3K9ac and H3K14ac, modifications characteristic of transcriptionally active euchromatin. Correspondingly, levels of H3K9 methylation are reduced. Chromatin is altered at multiple genomic loci; acetylation is upregulated in both active genes and constitutive heterochromatin. Likewise H3K9me3 and HP1α are reduced at pericentromeric foci and throughout the nucleus. Changes in both H3K9ac and methylation coincide with S-phase, suggesting for the first time a link between H3K9ac and replication in higher eukaryotes. SMARCAD1 localises to replication foci during S-phase and interacts both with replication factors and proteins associated with gene silencing. This suggests a mechanism whereby SMARCAD1 remodels newly replicated chromatin, facilitating histone modification and ensuring the fidelity of epigenetic inheritance.

IMAGING SINGLE PROTEIN MOLECULES IN LIVE EUKARYOTIC CELL NUCLEUS

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Within a single cell, a gene of interest exists only in one (or two) copy, and only a few protein molecules will interact with and control the regulatory region, making the decisions on the expression of the gene. Consequently, single-molecule experiments in individual cells are essential for the mechanistic understanding of eukaryotic gene regulation. We present a novel strategy using multifocal multiphoton microscopy to observe single fluorescent proteins fused to protein of interest in a live eukaryotic cell nucleus. To accomplish this, we extend the idea of single molecule detection by localization, which was successfully used in bacteria, to eukaryotic cells. The idea is that a single fluorescent protein cannot be imaged when freely diffusing in the nucleus but can be detected above the autofluorescence background upon binding to the less mobile DNA or the nuclear membrane. We use a scanning two-photon fluorescence microscope in order to reduce the strong out-of-focus autofluorescence background of the cells. To circumvent the slow image acquisition speed associated with point scanning and the inherent movement of the chromosomal DNA, we employ a multifocal excitation in combination with CCD detection, which allows us to achieve single molecule sensitivity in real time. As a proof of principle, binding and unbinding of single molecules of YFP labeled transcription factor on chromosomal DNA is demonstrated.

RARB2 CHROMATIN INABILITY TO TRANSITION TO THE POISED STATE IS A COMMON DENOMINATOR OF SILENT AND ACTIVE RARB2 IN CANCER CELLS

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The retinoic acid receptor RARB2 is a prototypical direct target gene of the retinoic acid receptor RARA, and its transcription is triggered by retinoic acid (RA) binding to RARA, and recruitment of chromatin activator complexes. The biological function of RARB2 is to mediate the growth inhibitory action of retinoic acid (RA). In cells sensitive to RA, RARB2 chromatin undergoes dynamic transitions from an inactive to an active state in the absence or presence of RA, respectively. A common feature of cancer cells is the resistance to the growth inhibitory action of RA. Here we show that RA resistance can be traced to opposite chromatin RARB2 states: either a silent RARB2 chromatin state that can no longer reach an active state in response to RA, or a constitutively active state that can no longer reach an inactive state in the absence of RA. In both these cases the RARB2 chromatin is unable to reach the “poised” state being locked in a state that either prevents RARB2 transcription in the presence of RA, or in a state that sustains transcription in the absence of RA. RARB2 inability to transition to the poised state seems to be the common denominator of RA-resistant cells with opposite RARB2 chromatin states.

NOVEL LINK BETWEEN NUCLEAR SPECKLES FORMATION AND RAN-RANBP2 SYSTEM

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Precise gene expression regulation is critical to cellular differentiation and development. It is proposed that global and local gene expressions are regulated through nuclear context and aberrations of the nuclear morphologies often link to diseases. Nuclear speckle/interchromatin granule cluster is one of the most prominent nuclear domains and it is often found adjacent to the site of active transcription, or spatial clusters of coordinately regulated genes. It is thought to play a role in coupling transcription and pre-mRNA splicing. Factors in nuclear speckles become distributed diffusely in the cytoplasm in early mitotsis, and they begin to reassemble into specific structures called mitotic interchromatin granules (MIGs) during metaphase. Previous elegant studies reveal that the components and RNA polymerase II enter into daughter nuclei sequentially and are recruited to the structure, in a programmed fashion during late telophase, which may establish transcription initiation pattern in the following cell cycle stages. To find molecules involved in the step, we semi-systematically knock-downed candidate proteins by RNA interference and investigated morphologies of nuclear speckles. We found that depletion of Ran, RCC1, or RanBP2 lead to MIG-like structures remain in the cytoplasm in G1 cells. Complementation analysis showed that SUMO E3 activity of RanBP2 partially contributes to this step. Surprisingly, cells with the impaired nuclear speckles are capable of constant and induced transcription and pre-mRNA splicing. However, alternative splicing patterns were de-regulated. Our results suggest a novel link between Ran-RanBP2 system, gene expression regulation and the inner structures of the nucleus.

LIVE-CELL ANALYSES REVEAL HOW MULTIPLE REPLICONS ARE PROCESSED FOR REPLICATION AT INDIVIDUAL FACTORIES.

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DNA replication is coordinated temporarily during S phase and is organized spatially within the nucleus. Groups of replicons are processed in discrete nuclear sites, called replication factories, which are composed of DNA polymerases and other replication proteins. The organization of chromosomal DNA replication in an individual replication factory still remains unclear. In this study, we marked three replicons on chromosome VII in budding yeast with fluorescent proteins and observed their behaviour during replication by live-cell imaging. Our data suggest that adjacent replicons were not always replicated in the same factory, and grouping of replicons in factories was indeed different from cell to cell. The physical distance between adjacent replicons in the nucleus during G1 phase did not affect the grouping of replicons for replication. On the other hand, chromosomal distance between the replicons did affect the grouping; the probability that adjacent replicons were replicated in the same factory was significantly higher than that of pairs of more distant replicons. We also developed mathematical modelling to analyze this process in more detail. Finally, to understand the dynamics of replication factories at a more global level, we are studying how replication factories change their organization during S phase and how such organization is altered in various mutants that are involved in DNA replication initiation, checkpoint activation and sister chromatid cohesion.

FORMATION OF A CELL CYCLE DEPENDENT DYNAMIC HMGA1A/ORC/HP1ALPHA COMPLEX IN HETEROCHROMATIN

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Binding of the heterohexameric origin recognition complex (ORC) to DNA is the first step in eukaryotic DNA replication but what determines ORC origin binding is unknown. High mobility group proteins A1a (HMGA1a) and heterochromatin protein 1 (HP1α) are mobile chromatin proteins belonging to a dynamic network that modulates heterochromatin organization. We have identified HMGA1a as a potential chaperone, which can mediate replication competence if targeted to DNA. Here we demonstrate that HMGA1a has a dual interface to interact with ORC: The acidic C-terminus is sufficient for HMGA1a's association with ORC and the smallest subunit, Orc6, interacts independently with HMGA1a's AT-hooks. The data we present here suggest that Orc6 and Orc1-5 can bind independently from each other to DNA and confirm that ORC form a heterohexameric complex *in vitro* and *in vivo*, suggesting that chaperones such as HMGA1a might stabilize ORC:DNA interactions specifying origins in certain chromatin regions.

We have extended this study by analyzing the trimeric interaction between ORC, HP1α, and HMGA1a. Biochemical and microscopic experiments support a model of an HMGA1a/ORC/HP1 α complex whose formation in heterochromatin is promoted due to the interaction of its components. We propose a stepwise dynamic assembly of the HMGA1a/ORC/HP1α complex in heterochromatin and support that an interaction-dependent stabilization of multimeric complexes may be a common theme of highly dynamic chromatin proteins.

SPATIO-TEMPORAL ORGANIZATION OF DNA REPLICATION STRUCTURES AT SUPER-RESOLUTION WITH 3-DIMENSIONAL STRUCTURED ILLUMINATION MICROSCOPY (3D-SIM)

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DNA replication, similar to other cellular processes, occurs within dynamic macromolecular structures in the size range of tens to a few hundred, i.e. beyond the reach of conventional light microscopy. The technology of three-dimensional structured illumination microscopy (3D-SIM) allows multi-wavelength optical sectioning of biological samples with sub-diffraction resolution in all three spatial axes. We have used 3D-SIM to directly measure and compare the size and numbers of replication foci (RF) in mammalian cells. Quantitative analysis revealed an average size of 125nm that was conserved throughout S-phase independent of the labeling method, suggesting a basic unit of genome duplication. Moreover, the improved optical 3D resolution identified about 5-fold more distinct replication foci than previously reported, with very similar RF-numbers from early S through mid-to-late S phase. By pulse-chase-pulse experiments and combined immunofluorescence staining of the replication clamp PCNA we found postreplicative DNA being displaced from sites of DNA synthesis within only a few minutes reveling new insights into the timing of replication fork movement.
These results demonstrate the unique capability of 3D-SIM for the multicolor visualization and accurate measurements of nuclear structures at a level previously achieved only by electron microscopy and highlight the possibility of high-throughput, multispectral 3D analyses.

TISSUE-SPECIFIC NUCLEAR ENVELOPE PROTEINS CAN REDIRECT SPECIFIC CHROMOSOMES TO THE NUCLEAR PERIPHERY

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The nuclear envelope (NE) is a microenvironment that contributes to genome organization and correspondingly to gene regulation. Mammalian chromosomes and some specific genes have non-random positions within the nucleus that are tissue-specific and heritable. It was recently shown also that chromosomes can be directed to the NE by an affinity mechanism. However, endogenous NE proteins that play a role in tissue-specific chromosome organization have not been identified. We performed a proteomic analysis that identified several NE transmembrane proteins (NETs) with restricted tissue expression. Many of these were over-expressed in HT1080 cells carrying LacO amplifications in different chromosomes to screen for those that could influence the positioning of the LacO insertion with respect to the NE. Eight yielded a statistically significant increase in the incidence of the locus at the NE. This was not an artifact of generalized condensation of chromatin towards the periphery as overall DAPI distribution was undisturbed. The effect was independent of the LacO amplification as the chromosomes in which the amplifications had been inserted also moved to the periphery with expression of these NETs in the parent HT1080 cells lacking the LacO sequences. DNA array analysis indicates that many of the genes altered in regulation upon expression of the NETs were clustered on the chromosomes, suggesting that some aspect of specific regions of the chromosomes defines their affinity for NE proteins. Further research will be required to determine if these NETs directly interact with regions on the chromosomes or if they modify other NE components for such tethering interactions, but these data represent the first demonstration of NE proteins involved in the tissue-specific organization of chromosome positioning. Furthermore the finding of this effect only with very tissue-specific NETs suggests that other aspects of higher-order genome organization may only be found after identification of tissue-specific components.

THE INTERPLAY OF DNA SUPERCOILING AND CATENATION DURING THE SEGREGATION OF SISTER DUPLEXES: PROKARYOTES VS. EUKARYOTES

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DNA gyrase is a unique topoisomerase that is able to introduce negative supercoiling into DNA duplexes. Eukaryotes lack DNA gyrase and their major source for torsional stress is the wrapping of DNA around nucleosomes. In any case, supercoiling is supported only within covalently closed domains. The presence of a free end in just one DNA strand of a duplex allows the tension responsible for supercoiling to be released. Here we used two-dimensional (2D) agarose gel electrophoresis to show that after completion of DNA replication, the resulting sister duplexes are catenated and the degree of catenation increases significantly when Topo IV (in prokaryotes) or Top2 (in eukaryotes) are selectively inhibited. To accumulate catenanes, we used a pBR322 derivative in a strain of Escherichia coli where Topo IV carries a temperature sensitive mutation. In Saccharomyces cerevisiae we analyzed an episomal minichromosome in a strain with the top2-td degron system that allows depletion of Top2 prior to DNA replication. The results obtained indicated that in prokaryotes DNA gyrase progressively introduces negative supercoiling as the sister duplexes become decatenated. In eukaryotes, on the other hand, wrapping of DNA around nucleosomes occurs immediately behind the fork during replication, but the compensatory positive supercoiling generated is automatically released at the free ends of the nascent strands. Wrapping of DNA around nucleosomes acquires topological significance instantly once the nascent strands are ligated and each sister duplex becomes a closed topological domain.

PAIRING OF HOMOLOGOUS CHROMOSOME REGIONS CORRELATES WITH THEIR FREQUENCY OF MITOTIC RECOMBINATION

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Using an in vivo mouse assay we demonstrated that the major mechanism of loss of heterozygosity (LOH) in somatic cells is mitotic recombination (MR). To categorize the sub-chromosomal regions that manifest different frequencies of MR in mouse primary skin fibroblasts, we genotyped both STS and SNP markers and determined that MR on mouse chromosome 8 occurs non-randomly and demonstrates sequence composition, chromatin structure, and nuclear spatial organization-specific characteristics. Regions with high and low recombination rates showed marked differences in GC content and repetitive element distribution indicative of a distinctive chromatin structure. Since MR requires contact between homologous regions of chromosome pairs, we investigated if nuclear architecture regulates the frequency of MR. The nuclear spatial organization of chromosome territories and sub-regions was analyzed by fluorescence microscopy and 3D reconstruction and with computational measurement techniques. We found that association between homologous chromosomes 8 occurred in 19% of 1281 exponentially growing cells. High and low recombination regions were not uniformly distributed within the chromosome 8 nuclear territories, suggesting that they belong to different sub-chromosomal compartments despite cis proximity (e.g., <4Mb). Homologous chromosomal regions with high MR frequencies were paired (<0.6 μm apart) in ~4% of 1281 exponentially growing cells in which both chromosome 8 territories were associated. This pairing was significantly greater ($p<0.001$) than that observed for homologous regions with low MR (0.3%). Pairing of homologous regions appears to be a directed event not accounted for by random variation of distances between chromosome territories during the cell cycle. Our results suggest that the physical closeness of homologous chromosomal regions may be the major factor governing the probability of a mitotic recombination event and support the hypothesis that nuclear architecture regulates the frequency of MR that results in distal LOH that may lead to tumorigenicity. Indeed, regions that manifest a high degree of MR on chromosome 8 also demonstrate elevated LOH in mouse mammary carcinomas and, LOH in syntenic human chromosomal regions is consistently associated with colon, breast and prostate cancers.

(*equal contribution)

THE IN VIVO TRANSCRIPTIONAL KINETICS OF SINGLE ALLELES REVEALS PROMOTER REGULATION DURING THE CELL CYCLE

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Eukaryotic gene expression is a complex and highly regulated process involving numerous steps, and is challenging to explore within living cells. Transcriptional kinetics have been measured in living mammalian cells, yeast, Dictyostelium, and E.coli. While in previous mammalian systems tandem gene-arrays were utilized to probe transcription site kinetics, such gene-arrays could not portray the true situation of single-allele genomic structure. We designed a system for visualization and analysis of mammalian mRNA transcriptional kinetics of single alleles in real-time, using a single-gene integration approach. High resolution measurements of a single cyclin D1 allele under either endogenous or viral promoter control were quantified. We provide analysis of the temporal kinetics of transcriptional bursting during the cell cycle, namely the frequency of promoter firing, the number of mRNAs present on the active gene, and the rates of transcription, on single genes in vivo. Post-replication transcription on both sister chromatids was identified as the newly replicated DNA began transcribing. Quantification demonstrated the dynamic reduction occurring in transcriptional activity efficiency after DNA replication. This analysis provides fundamental insights into the underlying cellular mechanisms that control gene expression during the cell cycle.

WHEN LESS IS MORE: USING UNDER-REPLICATED REGIONS TO PROBE ORIGIN FIRING DURING DNA REPLICATION

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During each eukaryotic cell cycle, each cell utilizes only a subset of its origins to initiate DNA replication. One of the difficulties confounding the study of metazoan origins is that very few have been molecularly defined, and little is known about what determines whether or not an origin is used or shut off for replication. In polyploid cells, cells with more than the diploid copy number of DNA, differential DNA replication can occur. For example, heterochromatin in many polyploid tissues is known to be under-replicated relative to the rest of the genome. We are using under-replicated euchromatic, gene-containing regions as models to define regulatory controls for metazoan origins. We have utilized comparative genomic hybridization (CGH) analyses to molecularly identify euchromatic regions that are under-replicated in polyploid and polytene tissues in Drosophila. In the salivary gland, we identified eleven prominently under-replicated regions, spanning several hundred kb, that reach relative ploidy levels of up to 10-fold under-replicated relative to the rest of the polyploid genome.

We found that under-replicated regions are tissue-specific, as different genomic loci are under-replicated in different larval and adult Drosophila polyploid tissues. Under-replication begins in the salivary gland from the first endo cycle, and correlates with a parallel reduction in transcription of genes from these regions during development. SuUR-/- mutant flies, which lack the Suppressor of Under-Replication protein, previously known to control under-replication of the heterochromatin, show full replication throughout the genome, as analyzed by CGH array. We are currently using ChIP-qPCR and ChIP-chip to map the binding of key replication proteins in the vicinity of under-replicated regions, to glean insight on the mechanisms by which replication fork progression or origin firing may be blocked at these loci.

GENOME WIDE CHROMATIN CROSS-TALK WITH MOUSE *H19* IMPRINTING CONTROL REGION REGULATES GENOMIC FUNCTION

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The genome forms extensive and dynamic physical communications with itself via long-range chromatin interactions. Recent evidences highlight that the complexity of nuclear architecture and three-dimensional genomic network contribute critically to the regulation of genomic functions. The causal link between such chromatin cross-talk and epigenetic states is, however, poorly understood. Using systematic high resolution 4C (Circular Chromosome Conformation Capture) analysis, we documented a comprehensive genome-wide screen of chromosomal interactions of mouse *H19* imprinting control region (ICR). The features of the interactions such as non-random positional clustering and association with transcriptional units reveal the contribution to the expressivity of the genome. In addition, the interactors' association with genes which show higher transcriptional activity during ES cell differentiation uncovers a growth related chromosomal network. Moreover, among all genome-wide interactions, we identified a network of physically juxtaposed regions from the entire genome with the common denominator of being genomically imprinted. CTCF-binding sites within the *H19* ICR not only determine the physical proximity among imprinted domains, but also transvect allele-specific epigenetic states, identified by replication timing patterns, to interacting, nonallelic imprinted regions during germline development. The observation suggests that one locus can directly or indirectly pleiotropically influence epigenetic states of multiple regions on other chromosomes with which it interacts. It also implicates that the three-dimensional organization of the genome plays important roles in regulating the genomic functionalities including transcription, replication and DNA repair in response to environmental changes during development, aging and disease.

MOLECULAR MECHANISMS OF THE REGULATION OF *RAX* EXPRESSION BY THE INNER NUCLEAR MEMBRANE PROTEIN NEMP1 IN VERTEBRATE EYE DEVELOPMENT

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To clarify the molecular mechanisms of early brain development, we have been analyzing the nuclear membrane protein gene *nemp1*, which is expressed in the *Xenopus* neural plate. Nemp1 has evolutionarily conserved region A in the transmembrane domains and region B in the C-terminal region, but not any known domains. The previous studies have shown that Nemp1 is localized to the inner nuclear membrane (INM) with region B facing the nucleoplasm, and that both over-expression and knockdown of Nemp1 in *Xenopus* embryos reduces the expressions of early eye-specific genes, *rax*, *pax6*, and *tbx3*, resulting in severe eye defects (Mamada et al., 2009, Dev. Biol. 327:497). Deletion analysis of Nemp1 has shown that biological activity of Nemp1 requires its nuclear envelope localization and region B, which was found to bind to the nuclear protein BAF through a consensus BAF-binding site. In this study, to reveal the molecular mechanism of Nemp1 functions, we analyzed Nemp1-target genes and sought Nemp1-interacting proteins. First, we showed that Otx2-induced *rax* expression in animal caps is inhibited by over-expression of Nemp1. Consistent with this, luciferase reporter assays showed that over-expression of Nemp1 inhibits activation of the *rax* CNS1 enhancer by Otx2, suggesting that Nemp1 regulates the *rax* gene through Otx2. Chromatin immunoprecipitation (ChIP) assays showed that Otx2 binds to the *rax* CNS1 enhancer at the early neurula stage when Nemp1 affects *rax* expression. Next, we analyzed Nemp1-interacting proteins, and found that Nemp1 interacts with Nemp1 itself but not with other inner nuclear proteins MAN1 and Emerin. Using yeast two-hybrid screening with region B as bait, we identified the small GTPase Ran. GST-pull down and coimmunoprecipitation assays showed that region B directly binds to a GTP form mutant of Ran, Q69L, but not to a GDP form mutant, T24N, or an effecter domain mutant, T42A. As expected, Nemp1 is co-localized with Ran at the nuclear envelope in transfected COS7 cells. In addition, in vitro binding assays showed that the interaction between GST-RanGTP and importin beta is inhibited by recombinant region B. These data lead to the possibility that Nemp1 is involved in the transcriptional regulation of the eye-specific gene *rax* through Otx2 by interacting with BAF and RanGTP at the INM.

FOCAL LOCALIZATION OF MUKBEF CONDENSIN ON THE CHROMOSOME REQUIRES THE FLEXIBLE LINKER REGION OF MUKF

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Condensin complexes are the key mediators of chromosome condensation. The MukB-MukE-MukF complex is a bacterial condensin, in which MukB subunit forms a V-shaped dimeric structure with two ATPase head domains. MukE and MukF together form a tight complex, which binds to MukB head via the C-terminal winged-helix domain (C-WHD) of MukF. One of the two bound C-WHDs of MukF is forced to detach from two ATP-bound, engaged MukB heads, and this detachment reaction depends on the MukF's flexible linker preceding the C-WHD. While MukB is known to focally localize at particular positions in cells by an unknown mechanism, mukE- or mukF-null mutation causes MukB to become dispersed in cells. Here, we report that mutations in MukF causing a defect in the detachment reaction interfere with the focal localization of MukB, and that the dispersed distribution of MukB in cells correlates directly with defects in cell growth and division. These data strongly suggest that the MukB-MukE-MukF condensin forms huge clusters through the ATP-dependent detachment reaction, and this cluster formation is critical for chromosome condensation by this machinery. We also show that the MukF's flexible linker is involved in the dimerization and the ATPase activity of MukB head.

POLYCOMB INFLUENCES THE NUCLEAR ORGANIZATION OF MULTIPLE GENES INTO DISTINCT EPIGENETIC DOMAINS IN MOUSE EMBRYONIC STEM CELLS

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The Polycomb complex and histone epigenetic marks are critical for controlling gene expression in embryonic stem cells (ESCs). Higher-order structures of chromatin, such as chromatin loops and 3D clustered genes, also influence gene expression. However, how epigenetic state at the molecular level translates into the nuclear organization of ESC chromatin is poorly understood. We investigated the nuclear organization of multiple linked genes according to their epigenetic state in ESCs. These included transcriptionally active genes marked, for example, with histone H3 lysine 4-trymethyl (H3K4-me_3), and so-called “bivalent” genes, which typically are repressed and contain both the active and repressive marks H3K4-me_3 and H3K27-me_3, respectively. Fluorescence in situ hybridization of multiple genes in the same cells revealed that active and bivalent genes are spatially separate from each other, forming epigenetically distinct micro-domains in ESC nuclei. Immunolabeling with antibodies against different covalent histone modifications further suggested the presence of distinct epigenetic domains throughout ESC nuclei. Differentiation of ESCs to mesoderm activated bivalent genes and resulted in their nuclear rearrangement, whereby they became part active gene domains. Consistent with this rearrangement being linked to epigenetic state, a null mutation in the Polycomb group gene Eed, which is required for H3K27-me_3, caused a similar nuclear redistribution of these genes. By super-resolution 4Pi imaging, we also found that active epigenetic domains at the nuclear periphery positioned closer to nuclear pores than to other structures within the nuclear envelope. Together, our data suggest a novel role for the Polycomb factor EED in genome higher-order structure, specifically in the nuclear organization of multiple genes into epigenetically defined micro-domains, a subset of which are structurally and potentially functionally coordinated with the nuclear envelope.

BALANCED RECIPROCAL TRANSLOCATIONS AS A TOOL TO STUDY GENOME ORGANIZATION

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Wilhelm Johannsen Centre for Functional Genome Research aims to identify novel genes by mapping the breakpoints within reciprocal translocations associated with disease phenotypes. Taking into account only 2% of the genome has been found to be associated with coding genes it is not so surprising that most of the translocation breakpoints fell into gene poor regions, unsequenced gaps or within segmental duplications pointing at potential disrupted regulatory mechanisms. As a model for studying the relationship between genomic rearrangements and chromatin reorganization, selected breakpoint regions will be presented where we have analysed the nuclear processes like transcriptional status and epigenetic marks by Next Generation sequencing and genome organization and replication status by fluorescence in situ hybridization based methods.

ALTERATION OF LEVELS OF THE SURVIVAL MOTOR NEURONS PROTEIN (SMN) AFFECTS NUCLEAR ARCHITECTURE IN DIFFERENTIATING NEUROBLASTOMA CELLS

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The protein Survival of Motor Neurons (SMN) is found in the cytoplasm,where it is required for the biogenesis of essential mRNA splicing factors termed snRNPs (small nuclear ribonucleoproteins). It is also found in the nucleus, where it localizes to nuclear bodies called gems. In most cultured cell lines, gems are indistinguishable from Cajal bodies (CBs), nuclear bodies with a role in the biogenesis and, probably, recycling of snRNPs. The close physical and functional relationship between CBs and gems is not fully understood. Insufficient expression of SMN results in the neurodegenerative disorder, Spinal Muscular Atrophy (SMA). It is not yet known whether the pathology of SMA results from defects in snRNP biogenesis with motor neurons being particularly sensitive to these defects, or whether the loss of an additional cell-type specific function of SMN may be the cause. We have investigated changes occurring within the nucleus during differentiation and neurite outgrowth in the human neuroblastoma cell line SHSY-5Y. CBs and gems increase their co-localization on differentiation, mimicking changes seen during foetal development. This change is associated with an increase in expression of endogenous SMN. Reducing the levels of SMN in SHSY-5Y cells disrupts the structure of both CBs and gems. We will present data concerning changes in nuclear architecture following differentiation of SHSY-5Y cells expressing different amounts of SMN.

INNER NUCLEAR MEMBRANE PROTEIN TRAFFICKING: MAPPING THE ROUTE OF MPS3

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Inner nuclear membrane (INM) proteins play an essential role in genomic organization and regulation of gene expression. The evolutionarily conserved SUN (for Sad1-UNC-84 homology) proteins play multiple roles in the three dimensional organization of the nucleus. In budding yeast, the sole SUN protein Mps3 is an essential component of the spindle pole body (SPB) and also localizes to the INM where it functions in establishment of sister chromatid cohesion, telomere tethering and regulation of certain types of DNA damage repair and recombination at the nuclear periphery. Critical to understanding how INM proteins function in establishment and preservation of nuclear organization is determining how they are targeted and maintained in the INM. Some proteins bound for the INM contain nuclear localization sequences (NLSs) that utilize an active transport pathway. However, many INM proteins, including Mps3, lack a classical NLS sequence and it is unclear how they come to reside and are retained in the INM.

Using single molecule imaging methods, the import of the non-NLS-containing INM protein Mps3-GFP was investigated to determine how it localizes to the INM. We examined the effects of disruptions in both passive diffusion and active transport pathways and deletions in certain nuclear pore complex subunits. Also, we determined sequences in Mps3 that are necessary and sufficient for nuclear import of a heterologous protein. Our data suggests that factors involved in active nuclear transport are required for retention of Mps3-GFP in the INM, although their function is most likely indirect through chromatin organization or assembly. The fact that chromatin-associated factors, including Sir2 and Sir4, along with the histone 2A variant H2A.Z (known as Htz1 in budding yeast), are essential for INM localization of Mps3-GFP further supports the hypothesis that retention of non-NLS-containing INM proteins such as Mps3 in the INM is an active, dynamic process.

OVEREXPRESSED TOPBP1 LOCALIZES TO NUCLEAR BODIES ASSOCIATED WITH PERIPHERY OF NUCLEOLI

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Human TopBP1 protein has multiple roles in cell cycle checkpoint, DNA damage response, DNA replication, transcriptional regulation and chromatin remodelling. It is essential for proliferation and participates in the initiation of DNA replication. During S phase of the cell cycle TopBP1 maintains genomic integrity through activating a vital DNA replication and stress response protein ATR (ATM and Rad3 related). Collectively, TopBP1 seems to promote pro-survival processes. TopBP1 is ubiquitously expressed in proliferating cells and localizes in nuclei. In primary breast cancer tissues, elevated levels of endogenous TopBP1 protein have been shown to correlate with aggressive cancer phenotype. This has been proposed to result from suppression of p53 activation and prevention of apoptosis.

TopBP1 protein levels seem to have an important role in regulation of its functions. Ectopic overexpression of an ATR-activating domain of TopBP1 activates cell cycle arrest and lead to senescence in cultured human cells. However, thus far the effects of full-length TopBP1 overexpression have not been studied in human cells. We have found overexpressed TopBP1 to accumulate in nuclear bodies, which associate with the periphery of nucleoli. Interestingly, a mutant TopBP1 incapable of activating ATR is retained from these bodies and predominantly diffuse staining of the nucleus is observed. We have generated a stable cell line inducible for expression of wild-type EGFP-TopBP1. We are in the process of characterizing the effect of TopBP1 overexpression and the nature of TopBP1 bodies.

INNATE IMMUNE RESPONSE IN SWINE: EXPRESSION AND NUCLEAR ORGANISATION OF TARGET GENES IN MACROPHAGES.

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Our project aims at a better understanding of the innate immune response of piglets by investigating the regulation of immune genes at both genomic and structural levels. Macrophages represent one of the first immune defences towards pathogens and their activities are highly regulated. The first release of the swine genome assembly is now available as genomic tools for immune response in this species. A DNA microarray targeting a set of 13297 pig genes and enriched with 3773 genes of the porcine immune system (Swine Leukocyte Antigen complex and immune genes) has been set up. The first aim was to analyse gene expression of porcine macrophages in different states (quiescent and LPS-stimulated to mimic bacterial infection) using this DNA array to get a better insight on the regulation of immune genes. Several authors have shown that nuclear localisation of genes also participates to their regulation. The second aim is to investigate if a differential expression of target genes induces modifications of their positions and their nuclear environment. The DNA array analysis reveals that more than sixty genes (qvalue<0.05) are differentially expressed when macrophages undergo activation and in the majority of cases over-expressed. The five more over-expressed genes and the four more down-regulated ones were selected and their expression levels were confirmed by RT-qPCR. These 9 Genes and their respective chromosome territories are investigated by 3D Fluorescent *in situ* Hybridization to study their nuclear localization in quiescent and activated macrophages. Using a dedicated software (Iannuccelli et al., Bioinformatics 2010), several 3D measurements were performed and allowed us to determine the size of the chromosome territories, the radial positioning of genes, and the distance between gene centers and chromosome edges. Differences of position in their chromosome territories were observed for some target genes in LPS-stimulated macrophages. We also studied the nucleus organisation of a particular region of interest for the immune response on porcine chromosome 7 (p1.1-q1.1) as it harbors the MHC complex.

DYNAMIC NUCLEOLAR FUNCTION OF RNA HELICASE DBP4 AND ASSOCIATED FACTORS.

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Ribosome biogenesis in the nucleolus is a highly complex and coordinated process that involves rDNA transcription, pre-rRNA maturation, and assembly of rRNAs with ribosomal proteins; the final maturation steps occur in the nucleoplasm and the cytoplasm to generate the small (40S) and large (60S) ribosomal subunits. Thus, many pre-ribosomal complexes are formed in this dynamic pathway.

The major steps in ribosome synthesis are conserved throughout eukaryotes. In yeast, more than 70 small nucleolar ribonucleoproteins and 170 non-ribosomal proteins (including different endo- and exonucleases, and 19 putative RNA helicases) participate in the assembly of the ribosome. Dbp4 is a phylogenetically conserved DEAD-box RNA helicase that is essential for yeast viability. In fact, Dbp4 is required for the early cleavage reactions that lead to the production of 18S rRNA.

We found that Dbp4 contains a predicted coiled-coil motif near its C-terminus. Because this motif is implicated in protein-protein interactions, Dbp4 might function in a complex with other protein(s). We identified a number of potential partners of Dbp4. Here we show that nucleolar proteins Bfr2 and Enp2 interact with Dbp4 in the two-hybrid system. Two-hybrid assay revealed that the C-terminal region of Dbp4 bearing the coiled-coil motif interacts with Bfr2 and Enp2. Immunoprecipitation experiments (IPs) further demonstrated that association of Dbp4 with Bfr2 and Enp2 is RNA-dependent, whereas association of Bfr2 with Enp2 is not. Various two-hybrid assays and IPs suggest that Bfr2 makes a bridge between Dbp4 and Enp2. We also found that, like Dbp4, Bfr2 and Enp2 are required for the early processing steps that lead to the production of 18S rRNA. Sucrose gradient sedimentation analyses indicate that Dbp4 co-sediments with Bfr2 and Enp2 in a wide peak of 40-60S. Bfr2, Enp2 and, to a lesser extent, Dbp4 were also detected in the 80S region of the gradient, suggesting a dynamic reorganization of large complexes that likely contain other nucleolar factors. In line with these observations, IPs showed that the stoichiometry of co-immunoprecipitated pre-rRNA species vary between Dbp4, Bfr2 and Enp2.

FUNCTIONAL RAD30 IS CRUCIAL FOR ESTABLISHMENT OF DAMAGE INDUCED COHESION GENOME WIDE IN BUDDING YEAST, WHICH IMPLICATES A NEW FUNCTION FOR THIS TRANSLESION SYNTHESIS POLYMERASE.

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By holding sister chromatids together from the moment of their formation during replication until their separation at anaphase, the multi subunit protein complex Cohesin guarantees correct chromosome segregation. This S-phase established chromatid cohesion is also essential for repair of DNA double strand breaks (DSBs) in postreplicative cells. In addition, it has been shown that Cohesin has to be recruited to a DSB, and new damage induced (DI-) cohesion formed in response to DSBs for efficient post replicative repair. Contrary to cohesion established during S-phase, DI-cohesion seems to be formed independently of DNA synthesis.
Rad30, (DNA polymerase η), is a translesion synthesis polymerase able to bypass UV-induced lesions with high fidelity. Rad30 has also been shown to efficiently extend D-loop substrates during homologous recombination (HR) and to be activated by the alternative RFC factor Ctf18, involved in both HR and cohesion establishment.
Using Saccharomyces cerevisiae as model organism we have investigated the importance of functional Rad30 for establishment of DI-cohesion. Despite that DI-cohesion is shown to be independent of DNA synthesis we found, to our surprise, that deletion of RAD30 or expression of a polymerase dead Rad30, as the sole source of Rad30, completely abrogates formation of DI-cohesion. Loading of Cohesin both to the break and genome wide is however unaffected. In addition Rad30 deficient cells show no defect in repair of DSB induced in G2. This suggests that the role of Rad30 in DI-cohesion is restricted to reactivation of the cohesion establishment machinery genome wide. This also reopens the question on the functional importance of genome wide DI-cohesion.

CHILL: CHROMATIN IMMUNO-LINKED LIGATION NOVEL AND VERSATILE TECHNIQUE FOR HIGH SENSITIVE ANALYSIS OF DNA-PROTEIN INTERACTION IN VIVO

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A chromatin immunoprecipitaion (ChIP) is a powerful technique to study DNA-protein interactions. As micrograms of the chromatin DNA is required for each assay, this poor sensitivity is a common drawback to ChIP, ChIP on chip and ChIP-seq assays. This is the sever limitation for scientists want to examine the rare cell sample or when the same analysis has to be performed for more than one protein of the interest. Moreover, low-resolution issue caused by mechanical chromatin sharing (sonication) that is often encountered in regular ChIP method that is cause of the problem to identify the protein binding sequence or quantify the binding efficiency in the region that has a series of binding site of same protein. Here we suggest the new technique, Chromatin immuno-linked ligation (ChILL) that overcomes these limitations. Outline of ChILL protocol is follows. An antibody of the choice conjugated with single strand DNA of 22 bp (named connecter DNA) is annealed with DNA fragment that has restriction site at the terminal (named reporter DNA). This nucleoproteic probe is added to crosslinked and restriction enzyme treated chromatin sample, upon the interaction of nucleoproteic probe with its target, the reporter DNA is ligated enzymatically to target DNA. The resulting product is de-crosslinked and purified. Finally the target DNA is mapped and quantified with PCR. This technique is easy to apply to high through put sequencing technology (Solexa sequencing) for genome wide analysis with incorporating the Solexa adapter sequence in reporter DNA. The principle of ChILL has already confirmed against insulator protein CTCF on its typical binding sites in the genome (H19/ICR and upstream of c-myc promoter) using human colon cancer cell line. Currently, ChILL is succeeded with hundreds of the cell as starting material compatible with high resolution (~250 bp).

OPERATOR BASED GENE TAGGING SYSTEMS (LACO/GFP-LACI OR TETO-GFP-TETR) REVEAL A NEW PATHWAY TO INDUCE GENE SILENCING IN BUDDING YEAST

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The spatial arrangement of genomes within the nucleus has emerged as a key regulator of their functions. The dynamics of individual genomic loci has been extensively studied by integrating protein-binding sites in the genome of cells expressing the corresponding fluorescent DNA binding proteins. We showed that both LacO/LacI and TetO/TetR gene tagging systems, can cooperate with cis-acting elements to induce gene repression and change in localization in budding yeast. Moreover, a LacI variant (LacI**) with a weakened affinity for its operator restores normal gene expression and localization of the tagged locus.
Thus, the recruitment of silencing factors by proteins tightly bound to DNA appears as new mechanism for silencing establishment. We are currently pursuing a genetic approach combined with live cell microscopy to determine the mechanisms by which the LacOp/GFP-LacI tagging system cooperates with silencers to establish silencing at a non-telomeric locus.

CLUSTERING HETEROCHROMATIN: SIR3 PROMOTES TELOMERE CLUSTERING INDEPENDENTLY OF SILENCING IN BUDDING YEAST

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A general feature in the nucleus of eukaryotic cells is the organization of repetitive DNA in clusters concentrating silencing factors. We investigated, in budding yeast, how telomeres cluster in perinuclear foci associated with the silencing complex Sir2/Sir3/Sir4. Here we show that Sir3 is a limiting factor for telomere clustering that when overexpressed leads to the grouping of telomeric foci into larger foci. These telomere “hyperclusters” are mainly found in the nuclear interior and correlate with more stable silencing in subtelomeric regions. However, we identified alleles of SIR3 that separate silencing and clustering function of Sir3. This latter function of Sir3 was effective in the absence of Sir2 and Sir4 but required the C-terminal domain of Rap1 responsible for recruiting Sir3 to telomeres. We thus demonstrate that Sir3 promotes telomere clustering in absence of perinuclear anchoring and independently of silencing. Furthermore, arrays of binding sites for Sir3 at telomeres appeared as the only requirement to promote trans-interactions between telomeres. We propose that similar mechanisms involving proteins able to oligomerize account for long-range interactions that impact genomic functions in many organisms.

GENETIC ANALYSIS OF THE MAINTENANCE OF UNDIFFERENTIATED STATE IN MOUSE ES CELLS

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Embryonic stem (ES) cells remain undifferentiated and are infinitely expandable as long as they are grown under appropriate conditions. In order to understand the underlying cellular mechanisms, we have developed a genetic screening system for selecting and analyzing autosomal recessive mutants in mouse ES cells.
We first mutagenized ES cells with a gene trap vector in a genome-wide manner, and transiently disrupted Bloom DNA helicase (Blm) gene by tetracycline-treatment, which induces mitotic recombination between homologous chromosomes and generates homozygous mutant daughter cells from heterozygous mothers. The homozygous mutant ES cells were then isolated from the heterozygous mutant background by a newly-developed selection system, and archived to create a library resource (manuscript submitted).
Interestingly, among hundreds of heterozygously mutated alleles in the above process, it turned out that almost one third were incapable of yielding corresponding homozygous viable alleles, suggesting that complete knockout of some of these genes, which included several genes involved in regulation of nuclear organization and function, could have seriously affected the maintenance of undifferentiated and proliferative state of mouse ES cells. To address the possibility, we are generating ES cells carrying conditional knockout alleles for the candidate genes, which will allow us to explore key cellular components required for ES cell maintenance.

SPATIOTEMPORAL REGULATION OF ACTIVITY-DEPENDENT GENES IN POST-MITOTIC NEURONS.

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An increasing body of evidence shows that sub-nuclear spatial gene positioning is of great relevance in a wide range of cellular functions such as differentiation and gene expression. For instance, the nuclear periphery has been shown to constitute a repressive environment for gene transcription in mammalian cells. Neurons express a number of specific genes upon depolarization in response to external stimuli. Although signaling pathways and transcription factors involved in activity-dependent gene expression in neurons have been intensively studied, spatial positioning and chromatin regulation of activity-dependent neuronal genes remain elusive. We have mapped the sub-nuclear spatial positioning and chromatin states of activity-dependent genes in mouse hippocampal neurons and delineated their spatio-temporal regulation. Using microarray analysis we have identified sets of genes which are upregulated within 30 minutes (designated as the early genes) while another set of genes is upregulated around180 minutes (the late genes) after depolarization . DNA fluorescence *in situ* hybridization revealed that the late genes are preferentially located at the nuclear periphery while the early genes are not. Surprisingly the late genes were transcribed at the periphery after depolarization, indicating the nuclear periphery in neurons is a transcriptionally permissive environment. The late genes are enriched in a repressive histone marker, di-methyl lysine 9 of histone H3, and get phosphorylated on serine 10 of H3 upon depolarization. The early genes are enriched in promoter-initiated RNA polymerase II (RNAP II) phosphorylated on serine 5 of its C-terminal repeat domain. Negative elongation factor (NELF) also occupies the proximal region of the early genes and plays a critical role in RNAP II stalling. These results demonstrate that the temporal regulation of activity-dependent genes in post-mitotic neurons correlates with the sub-nuclear spatial positioning and chromatin states.

MECHANISM OF DNA DAMAGE-INDUCED FOCUS FORMATION OF RAD51

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DNA damage-induced foci are higher order nuclear architectures formed by the accumulation of proteins associated with DNA repair at sites containing DNA damage. Posttranslational protein modifications, such as phosphorylation, acetylation and ubiquitination, have been suggested to play a role in the regulation of protein dynamics. Among protein modifications, covalent binding of SUMO-1 to target proteins, or SUMOylation, is implicated in the formation of PML bodies, the most prominent non-chromatin nuclear domains. However, little is known about the role of SUMOylation in the DNA damage-induced focus formation. We previously found that RAD51, a key protein involved in the recombinational repair of DNA double strand breaks (DSBs), forms nuclear foci during S phase, and also accumulates at sites containing DNA damage. RAD51 is known to interact with SUMO-1. Here we show that SUMO-1 is required for the recruitment of RAD51 at damaged sites. We also found that interaction of RAD51 with SUMO-1 is essential for the RAD51 accumulation at sites containing DSBs. These findings suggest the involvement of SUMO modification system in the DNA damage-induced focus formation of RAD51. Regulation of DNA damage-induced nuclear domain formation by protein modifications will be discussed.

PIN1 REGULATES NUCLEAR LOCALIZATION AND UBIQUITINATION OF A SUBSET OF RNA PROCESSING FACTORS

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Proteins involved in various stages of posttranscriptional control of gene expression are regulated by reversible posttranslational modifications. These modifications include phosphorylation, acetylation, lysine and arginine methylation, sumoylation and ubiquitination. Frequently these posttranslational events are inter-linked, yet the role(s) of various modifications in RNA recognition and the mechanism(s) by which they influence gene expression are not well understood. Prolyl isomerases can act as molecular chaperones as well as regulators of the activity of proteins involved in cell signaling and transcription by catalyzing rapid conformational changes in their substrates via prolyl cis-trans isomerization. The prolyl isomerase Pin1 acts specifically on Ser/Thr phosphorylated targets, helps to regulate mitosis, and is overexpressed in a number of cancers. Pin1 has previously been implicated to control the transcription cycle by regulating the phosphorylation state of the RNA polymerase II C-terminal domain (CTD). The yeast orthologue of Pin1, Ess1, has also been implicated in transcription initiation and termination by enhancing gene looping. We will present results from ongoing studies that show that phosphorylation-specific prolyl isomerization directed by Pin1 regulates the histone mRNA specific RNA processing factor SLBP and the stability of the SLBP-histone mRNA complex in vitro. RNAi knockdown of Pin1 in Hela cells results in increased SLBP protein stability and increased accumulation of SLBP in the nucleus. Pin1 acts with PP2A to regulate SLBP ubiquitination. To determine whether Pin1 plays a general role in control of RNA-mediated gene expression, we screened a number of RNA processing factors and examined the effects of RNAi knockdown on their stability and localization. The results indicate that two subunits of CPSF are also Pin1 targets. Results from these ongoing studies will be presented.

PROPER NUCLEAR ENVELOPE STRUCTURE REQUIRES THE RSC CHROMATIN-REMODELING COMPLEX

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An intact nuclear envelope (NE) imbedded with properly assembled nuclear pore complexes (NPCs) is required for sustained events of transcription, replication, genome maintenance and nucleocytoplasmic transport. Therefore, it is a high priority to identify the underlying mechanisms that support the biogenesis and integrity of the nuclear periphery. We employed two independent screening strategies to identify mutants with defects in NPC localization and NE structure. Collectively, we found that multiple components of the RSC chromatin-remodeling complex are required to properly localize GFP-tagged nucleoporins. Electron microscopy studies further resolved that the nucleoporin mislocalization patterns observed reflected alterations in NE structure. This included extensive sheets and intertwined honeycombs of membrane, often making it difficult to identify the nucleus. Furthermore, these alterations occurred independent of cell division and required ongoing transcription and translation. Two additional observations provided insight into the cascade of events preceding the NE alterations in RSC mutants. First, increasing membrane fluidity with benzyl alcohol treatment prevented the NE structural defects and NPC mislocalization in a temperature arrested mutant allele of the RSC ATPase, *sth1-F793S*. Second, osmotic cushioning with sorbitol suppressed both the nuclear pore complex mislocalization and temperature sensitivity of *sth1-F793S*. We speculate that RSC is required to preserve global chromatin architecture and thereby assists in the proper execution of signaling events and transcriptional programs that preserve NE structure.

A CLASSICAL NLS, A GOLGI RETRIEVAL SIGNAL AND THE SUN DOMAIN CONTRIBUTE TO THE TARGETING OF SUN2 TO THE INNER NUCLEAR MEMBRANE

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Inner nuclear membrane (INM) proteins are co-translationally inserted into the endoplasmic reticulum membrane (ER) and need to cross the nuclear pore complex (NPC) to reach their final destination. Originally, targeting of INM proteins to the INM was solely explained by a “diffusion-retention” model. However, recent studies on yeast INM proteins revealed the existence of classical nuclear localization signals (cNLS) that mediate the active transport of the proteins across the NPC. Whether active transport mechanisms also play a role in the targeting of INM proteins in mammalian cells is still unclear. We have used human SUN2 to study INM targeting and identified three different elements, which collectively mediate NE targeting. The N-terminal nucleoplasmic domain of SUN2 comprises a cNLS and a Golgi retrieval signal. The cNLS was shown to constitute a functional binding site for the heterodimeric transport receptor importin α/β. A nearby arginine cluster was found to serve as binding platform for the coatomer complex, which mediates retrieval of SUN2 from the Golgi to the ER and ensures efficient INM targeting. The conserved SUN domain at the C terminus represents the third targeting element we have identified in our study (see abstract Rothballer). Together, our study shows that multiple elements in SUN2 contribute to NE targeting and that these elements are not limited to cytoplasmic or transmembrane domains.

CROSS-TALK BETWEEN HISTONE ACETYLATION AND METHYLATION DURING DNA DOUBLE STRAND BREAK REPAIR

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DNA is a constant target of exogenous and endogenous hits which lead to variety of DNA lesions. Among these lesions, double strand breaks (DSB) are considered the most deleterious, therefore cellular defects in DNA damage response and DNA repair inevitably results in deregulation of critical cellular processes and human diseases, most notably cancer. Recent studies revealed that histone modifications play important roles in cellular response to DNA breaks and DNA repair, in addition to their classical involvement in transcriptional regulation. Recent work from our laboratory implicated histone acetylation in chromatin unwinding that facilitates the repair of DSBs [1-2]. However, precise order of molecular events and dependences among different histone modifications during DNA damage response and DNA repair remains poorly understood [4]. Here, we studied the cross-talk between histone acetylation and methylation after DNA damage using cells that allow deletion of specific histone modifiers. We found that deletion of Suv39h, methyltransferase responsible for H3K9 trimethylation, resulted in an increased acetylation of histones and accumulation of HAT on the chromatin. Suv39h deletion also impaired an enrichment of H3K9me3 after DNA damage and resulted in an altered kinetics of recruitment of DNA repair proteins including MDC1, 53BP1 and Rad51 and an aberrant DNA repair efficiency through homologous recombination. Perhaps surprisingly, we found that abrogation of HAT recruitment through depletion of HAT cofactor Trrap resulted in a decrease of H3K9me3 following DNA damage. Impairment of H3K9me3 marking prevented phosphorylation of downstream targets of ATM kinase, albeit without obvious effect on cell cycle progression and apoptosis. Together, these results suggest the existence of an intricate cross-talk and dependences between histone acetylation and methylation which regulate the kinetics of recruitment of DNA repair proteins and DNA repair, thus preventing genomic instability.

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THE INSULATOR PROTEIN SU(HW) MODULATES NUCLEAR LAMINA INTERACTIONS OF THE *DROSOPHILA* GENOME

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Specific interactions of the genome with the nuclear lamina are thought to assist chromosome folding inside the nucleus and to contribute to the regulation of gene expression. High-resolution mapping has recently identified hundreds of large, sharply defined lamina-associated domains (LADs) in the human genome, and suggested that the insulator protein CTCF may help to demarcate these domains. Here, we report the detailed structure of LADs in *Drosophila* cells, and investigate the putative roles of five insulator proteins in LAD organization.
We used DamID to generate high resolution genome wide binding profiles of the five *Drosophila* insulator proteins: Su(Hw), Beaf32B, CTCF, DWG and GAGA Factor. Computational analysis revealed that of these five proteins, only Su(Hw) binds preferentially at LAD borders and at specific positions inside LADs, while GAF, CTCF, BEAF-32 and DWG are mostly absent from these regions. To directly test the role of Su(Hw) in LAD formation we created a high-resolution map of genome - Lamina interactions after knockdown and overexpression of Su(Hw). These experiments demonstrate that Su(Hw) weakens genome – Lamina interactions through a local antagonistic effect. Our results provide insights into the evolution of LAD organization and reveal a role for Su(Hw) in chromosome organization within the nucleus by modulating genome - Lamina interactions.

FUNCTIONAL INTERACTIONS BETWEEN THE NUCLEOPORIN NUP170P AND CHROMATIN REMODELING FACTORS

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Nuclear pore complexes (NPCs) are evolutionarily conserved, multi-protein structures that extend across the nuclear envelope (NE) and control the movement of soluble transport factors and their attached cargos into and out of the nucleus. NPCs are comprised of ~30 proteins termed nucleoporins, each present in multiple copies. In addition to their role in regulating nuclear transport, it has become clear that NPCs perform a vital role in chromatin organization and the regulation of gene expression through mechanisms not directly related to transport. To gain further insight into the role of the NPC in these processes, we have investigated the function of the yeast nucleoporin Nup170p. Previous data has indicated that the loss of *NUP170* leads to chromatin instability. Moreover, in data that will be presented, we have shown that deletion of *NUP170* results in a loss of silencing in telomeric regions and the mating type loci, and aberrant regulation of *MAT*(a) specific genes. Several lines of evidence suggest that these phenotypes arise due to defects in NPC assembly caused by the loss of Nup170p. Further analysis of the *nup170* deletion mutant using genetic screens revealed a network of synthetic sick or lethal genetic interactions between mutations in *nup170* and genes encoding components of chromatin remodeling complexes. Importantly, analysis of several double mutants showed they contained fewer NPCs suggesting their growth deficiencies are linked to defects in NPC assembly. Moreover, we detected a physical link between Nup170p and Sth1p, a component of the RSC (Remodels the Structure of Chromatin) complex. Components of RSC share many of the same genetic interactions as Nup170p, but no genetic interactions have been detected between this complex and Nup170p. These data are consistent with the idea that they function in the same pathway, one distinct from the genetically interacting chromatin remodelers. Finally, as with the loss of Nup170p, conditional *STH1* mutations exhibit defects in NPC assembly, including the accumulation of similar NPC assembly intermediates. On the basis of these results, we hypothesize that Nup170p functions as a key component in the formation of the NPC and as a platform for the recruitment of chromatin remodeling complexes, in particular RSC, to sites of NPC formation. Here, we predict these proteins function in NPC assembly and in the formation, and/or maintenance, of chromatin domains in the vicinity of the NPC.

SIRT1 REGULATES SUV39H1 LEVELS AND INDUCES ITS UPREGULATION UPON OXIDATIVE STRESS CONDITIONS

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The Sir2 family constitutes the Class III of histone deacetylases. In humans, there are 7 Sir2 members or Sirtuins (SirT1 to 7), and reports have linked them to multiple mechanisms from stress survival and metabolic homeostasis to cell cycle control through deacetylation of histone and non-histone proteins. However, the conservation of this family through evolution suggests that in addition to newly acquired functions, Sir2 family have core functions that explain their presence up to mammals. One of these functions seems to be chromatin regulation. Among Sirtuins, only SirT1-3 and 6 have been shown to be histone deacetylases. In particular, SirT1 participates in the formation of facultative heterochromatin through the deacetylation of H4K16Ac and H1K26Ac, and a functional interaction with the H3K9me3-methyltransferase Suv39h1. The relationship between SirT1 and Suv39h1 goes beyond the expected interplay between HDACs and HMTs; SirT1 promotes the establishment of H3K9me3 by four different mechanisms: a) Deacetylation of H3K9Ac to allow methylation by Suv39h1, b) Direct recruitment of Suv39h1, c) Increase of Suv39h1 specific activity by a conformational change upon interaction and d) Deacetylation of K266 in the catalytic SET domain of Suv39h1, which renders a more active enzyme. The relationship between SirT1 and Suv39h1 is also key in constitutive heterochromatin (CH): Mouse embryonic fibroblasts (MEFs) derived from SIRT1-/- mice show a complete loss of H3K9me3 and HP1 in 50% of the MEFs analyzed. Until now, we have no clear explanation for this observation since SirT1 does not seem to localize to CH in any of the tested conditions.

Here we show a new mechanism through which SIRT1 induces Suv39h1 function that can explain the role of SIRT1 in CH formation: SIRT1 controls the global levels of Suv39h1 in vivo by inhibiting its degradation by the proteosome system. The mechanism involved requires the N-terminal domains of both SirT1 and Suv39h1 and increases at least three-fold Suv39h1 half-life. Interestingly, in vivo conditions that upregulate SirT1, such as Calorie restriction and other oxidative stress situations also induce higher levels of Suv39h1 in a SIRT1-dependent manner. This upregulation might trigger proliferation arrest and have important consequences in cellular senescence and chromatin integrity upon stress. Remarkably, these observations reflect the first direct link between oxidative stress response and a chromatin organization keystone such as Suv39h1.

CRM1 AND NOP58 SUMOYLATION CONTROL THE NUCLEOLAR LOCALIZATION OF BOX C/D SNORNP COMPLEXES

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CRM1 plays an important role in eukaryotic cells, by exporting numerous proteins and several RNPs. In this study, we have focused on the role of CRM1 in the transport of Box C/D snoRNA to nucleoli. Indeed, CRM1 was shown to be required for this process, but its mechanism of action was unclear. We showed that, surprisingly, CRM1 is not acting as an export receptor in this process. Instead, we found that CRM1 acts in the nucleoplasm, and regulates the binding of Tgs1, the sn/snoRNA cap hypermethylase, to Box C/D snoRNP. The Tgs1-snoRNP interaction seems to involve Nop58, one of the core protein of Box C/D snoRNP. This protein possesses a C-terminal domain rich in charged amino acids that interacts with Tgs1 in vitro and that functions as a nucleolar localization signal (NoLS). Thus, when CRM1 is inactivated, Tgs1 would bind the NoLS of snoRNPs and prevent their transport to nucleoli. This is the first example of CRM1 playing a role in RNP intranuclear transport by modifying RNP composition in interphase nuclei.
We also found that the C-terminal domain of Nop58 is SUMOylated, and we showed that a non-sumoylable mutant of this protein has a reduced affinity for snoRNAs. Whether SUMOylation of Nop58 is important for assembly and/or transport of snoRNPs to nucleoli is under investigation.

RECOMBINATION-INDUCED TAG EXCHANGE REVEALED RAPID REPLICATION-INDEPENDENT TURNOVER OF CANONICAL HISTONES

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Recent studies have shown that not only histone modifications, but also histones themselves can be highly dynamic, suggesting that epigenetic marks are not permanent at a given genomic location. To better understand the dynamic nature of chromatin, we developed a new *in vivo* assay in budding yeast that discriminates between existing and newly synthesized proteins, called RITE for Recombination-Induced Tag Exchange. The advantage of RITE is that it makes use of the endogenous promoter of any gene of interest and can be used under physiological conditions without any chemical perturbations to the cell.

We applied this universally applicable tool to histone H3 to study for the first time the dynamics of canonical histones regulated by the endogenous promoter. Even though histone expression peaked during S-phase when chromatin is duplicated, we observed global deposition of new canonical histone H3 proteins outside of S-phase throughout the cell cycle. In addition we found a positive correlation with transcription levels and that transcription can enhance exchange. A direct comparison with replication-dependent histone deposition showed that cells can replace half of the old chromosomal histones by new ones in only a few hours by replication-independent mechanisms. The high rate of histone exchange suggests that epigenetic marks are regularly being reset.

We are currently using RITE to determine how histones are inherited in dividing cells. In addition we are combining RITE with the yeast knock-out collection to screen for mutants affecting histone exchange to unravel the underlying mechanisms. We have already identified several genes that can promote or counteract histone exchange.

CHOOSING THE TARGET LOCI: CHIP-CHIP AND CHIP-SEQUENCING ANALYSIS OF HSF1 AND HSF2 TARGET GENES IN STRESS AND DEVELOPMENT

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Heat shock transcription factors (HSFs) are an evolutionarily well conserved protein family that protects cells from detrimental stresses and guides developmental processes. Upon proteotoxic stress, HSF1 and HSF2 bind to the promoters of heat shock protein (Hsp) genes, thereby activating transcription. The HSF-mediated heat shock response is a remarkable manifestation of transcription factors' capacity to rapidly locate and bind to the cell type- and stimulus-specific target loci. The importance of cell-specific transcriptional control, signalling cascades and nuclear organization is illustrated in spermatogenesis, where HSF expression is crucial for male fertility. Although being abundantly expressed in testis, HSF1 and HSF2 do not induce Hsp transcription in male germ cells. Using ChIP-chip approach, we have identified HSF1 and HSF2 target promoters in mouse spermatogenesis, revealing a profound HSF1 and HSF2 occupation on multicopy gene families that reside on the repetitive and palindromic sequences of X- and Y-chromosomes. Meiotic silencing of sex chromosomes has recently been reported to be partially reactivated after meiosis, leading to initiation of sex-linked multicopy gene transcription. Strikingly, we have found HSF1 to locate to sex chromatin both before and after meiosis, thereby regulating sex-chromosomal gene expression in the repressed environment. To further understand how nuclear organization influences the genomic distribution of HSFs, we are conducting a ChIP-sequencing experiment in synchronized human K562 erythro leukemia cells. By analyzing HSF1 and HSF2 target loci in interphase and mitotic cells, either under optimal growth conditions or upon stress, we will address how mitotic chromatin compaction affects the genomic distribution of HSF1 and HSF2. Our ultimate goal is to conduct a ChIP-sequencing experiment also in mouse testis. Revealing HSF1 and HSF2 target loci in human carcinoma cell line and in mouse testis will provide profound information on the plasticity of transcription factors in regulating gene expression.

NEW INSIGHT INTO RNA POLYMERASE II PAUSING

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The field of eukaryotic transcription has seen fundamental conceptual changes in recent years. Genome wide mapping studies revealed promoter proximal pausing of RNA polymerase II (RNAPII) at 30% of human genes, establishing post-initiation events as a globally important step in gene regulation. The carboxy terminal domain (CTD) of RBP1, the largest subunit of RNAPII has been proposed to serve as a binding scaffold for numerous factors, which in concert ensure proper maturation of the RNAPII complex through the transcription cycle from initiation, pausing to clearance, elongation and finally release from the template. Transition through these steps is tightly regulated. RNAPII pausing/recycling shortly after initiation is becoming a hallmark of gene regulation. However, the molecular mechanisms involved in RNAPII pausing/recycling are largely unknown.

The human immunodeficiency virus type 1 promoter is a well defined, convenient and thus widely used model, which has provided considerable insight into transcriptional elongation control. Transcription from the long terminal repeat (LTR) leads to RNAPII pausing after synthesis of a short RNA, the transactivation response element (TAR). Paused RNAPII becomes unstable and released from the DNA. The HIV-1 transactivator protein, Tat, together with Cyclin T1 binds a bulge-loop within TAR allowing CDK9 to phosphorylate the RNAPII CTD and NTEFs, licensing RNAPII for productive elongation. We will present evidence for the involvement of the microprocessor complex DROSHA and DGCR8, involved in microRNA biogenesis, in regulating RNAPII processivity and recycling. This newly identified function of DROSHA/DGCR8 is independent of RNAi and requires the presence of TAR RNA.

A-TO-I RNA EDITING IS REGULATED IN THE PRIMARY NEURONAL CELL CULTURE

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Many transcripts coding for genes involved in neurotransmission are subjected to A-to-I (adenosine to inosine) RNA editing. ADARs (adenosine deaminases that act on RNA) are enzymes responsible for this process. They target adenosines located within double stranded RNA that often are found within conserved regions of the transcript. Since inosine is recognised as guanosine by the translational machinery A-to-I editing can give rise to an amino acid change with profound effects on the encoded protein. We have recently shown that there is a global regulation on RNA editing during development of the mouse brain (Wahlstedt et al. 2009). The editing frequencies are in general low in the embryonic brain but increase dramatically during the early postnatal days. In mammals two ADAR enzymes (ADAR1 and ADAR2) have been shown to carry out the deaminase reaction. These enzymes have overlapping but specific target recognition. The low editing frequencies detected in the young brain cannot be explained by low expression of the ADAR proteins. We show that the level of ADAR protein in the brain is constant during development. To further investigate the regulation of editing during development, neurons from cortex of mice at embryonic day 18 (E18) was cultured for two weeks. In the primary cell culture the editing frequency of known targets follow the same pattern as detected in vivo. The editing levels increases with days in culture and after 12-14 days editing reach its maximum. Furthermore, we show that the level of ADAR proteins is constant in these cultured neurons indicating that there are other mechanism that control the developmental regulation of site selective A-to-I RNA editing. Other possibilities for editing regulation such as ADAR protein modification and cellular location will be discussed.

MOLECULAR INTERPLAY OF THE NON-CODING RNA *ANRIL* AND METHYLATED HISTONE H3 LYSINE 27 BY POLYCOMB CBX7 IN TRANSCRIPTIONAL SILENCING OF P16^{INK4A}

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Expression of the *INK4b/ARF/INK4a* tumor suppressor locus in normal and cancerous cell growth is controlled by methylation of histone H3 at lysine 27 (H3K27me) as directed by the *Polycomb* group proteins. The antisense non-coding RNA *ANRIL* that spans much of the *INK4b/ARF/INK4a* locus has also been suggested to play an important role in the regulation of opposing protein-coding genes in *cis*, but the underlying mechanism has remained elusive. In this study, we report that chromobox 7 (CBX7) within the Polycomb Repressive Complex 1 (PRC1) binds specifically to *ANRIL*, and both CBX7 and *ANRIL* are found at elevated levels in prostate cancer tissues. We demonstrate that in concert with H3K27me recognition, CBX7 binding to RNA is essential for p16^{INK4a} repression, and that disruption of either interaction leads to accelerated cell death and characteristic senescent morphology. Structure-guided analysis reveals the molecular interplay between non-coding RNA and H3K27me as mediated by a single conserved chromodomain. Our study suggests a new mechanism by which non-coding RNA participates directly in the dynamic regulation of epigenetic transcriptional repression.

HISTONE H2B REGULATES TELOMERE SILENCING INDEPENDENTLY OF H2B UBIQUITYLATION

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Eukaryotic genomes are packaged into nucleosomes. In the yeast nucleosomal core particle, the histone H2B C-terminal helix lies exposed on the surface of the histone octamer, and interacts with histone H3 of neighboring nucleosomes. The H2B C-terminus plays a critical role in chromatin organization. H2B Lys123 ubiquitylation, for example, promotes H3 lys4 and lys79 methylation and has been linked to transcriptional activation and silencing. However, we found that deletion of the H2B C- terminus (H2BΔC 122-130) exhibits a more severe growth defect than the ubiquitin-deficient mutant, H2B K123R, implying that other residues of the H2B C-terminus, besides K123, also play important roles in cellular function. Although there are several potential residues for phosphorylation in the H2B C-terminus; proteomic analysis revealed that H2B T128 alone is a potential phosphorylated residue. In order to understand the contributions made by the H2B C-terminus, we have systematically mutagenized the C-terminal residues between T122-T128. As seen for the K123R mutant, cells expressing a mutant form of H2B with T122 and Y124 replaced by glutamic acid (T122E & Y124E), were sensitive to caffeine and HU but not to MMS, suggesting its importance for regulating DNA replication. Moreover, the T122E and S125E mutants tended to elevate the cellular level of H2Bub1, but only T122E affected telomere silencing dramatically. The silencing defect of T122E, however, was more severe than that observed on either deletion of UBP10, which removes H2Bub1 from the telomeric regions, or in strains carrying an S120A substitution within ubiquitin conjugating enzyme Rad6 that diminishes H2B ubiquitylation, implying that T122E may regulate the silencing effect through a novel mechanism other than increasing H2Bub1. To identify the putative roles of the H2B T122 on heterochromatin conformation, we determined the distribution of the silencing-associated factors on chromatin in H2B T122A/E mutants. The distribution of several marks, including Sir2 and acetylated H4 K16, were changed in T122E mutants but not in T122A. Despite the elevated H2Bub in T122E, we did not observe an increase of trimethylated H3 K4 and K79 at telomeric regions. Overall, these data suggest that the charged residue at T122 may be essential to the organization of the telomeric chromatin. We propose that telomere silencing is not only controlled by the recruitment of Sir complex to the telomere as mediated by the levels of H2Bub1, but that the charged residue at T122 is perhaps important for nucleosome-nucleosome interaction and higher order-chromatin structure, in an effect independent of H2B ubiquitylation.

HOW DO THE CO-REPRESSOR HDACS ACT IN THE HUMAN GENOME

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Histone acetyltransferase (HAT) and deacetylase (HDAC) are two groups of enzymes with antagonizing function to control acetylation, which is critical for any organisms to precisely modulate gene expression, either activation or repression. Abnormality of this process may result in human diseases including cancers. As acetylation is a histone mark for active transcription, HATs, co-activators of transcription, have been associated with active and HDACs, co-repressors of transcription, with inactive genes, respectively. This so-called “On/off” model has been used to interpret data in numerous investigations.

Using ChIP-Seq, we mapped the genome-wide distribution of HATs and HDACs in the human genome and found that both bind to active genes with acetylated histones. The majority of HDACs, including Class I, II, and III HDACs, function to reset the chromatin by removing acetylation at active genes. Inactive genes that are primed by H3K4 methylation marks are subject to a dynamic cycle of acetylation and deacetylation by transient binding of HATs/HDACs, which prevents the Pol II binding but poises them for future induction. In contrast to the traditional “On/off” model, we found that constitutively silent genes without any H3K4 methylation signals show no evidence of being bound by co-repressor HDACs. We also found that the recruitment of HDAC6 and TIP60 to gene body is mediated through the interaction with elongating Pol II.

In summary, our discoveries provide new insights into the function of HATs and HDACs. The emerging new concept/functional role of co-repressor HDACs provides a new perspective to understand the mechanisms of HDAC inhibitors in treating human diseases.

EPIGENETIC ANALYSIS OF CHROMATIN DOMAINS AT HUMAN NEOCENTROMERES

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Human centromeres contain large amounts of tandemly repeated alpha satellite DNA, which contains both CENP-A/H3K4me2 centrochromatin and H3K9me3 heterochromatin. Neocentromeres are fully functional centromeres that form in ectopic chromosomal locations on low copy DNA, and do not require specific DNA sequences. Thus, neocentromeres provide the opportunity to analyze centromeric chromatin domain organization, which is not possible at endogenous centromeres because of the large amounts of highly homologous repetitive DNA. CENP-A ChIP analysis showed that neocentromeres contain ~100 kb of CENP-A chromatin in a two-domain organization. Additional CENP-A domains near neocentromeres coincide with CpG islands at the 5′ end of genes. Small domains of H3K4me2 are found within the CENP-A domains at neocentromeres, but these are also found in equivalent non-neocentric chromosomes. This suggests that H3K4me2 does not play a higher-order structural role at neocentromeres, as suggested for endogenous centromeres. A striking paucity of H3K9me3 was observed at neocentromeres. Thus, it appears that large domains of heterochromatin are not strictly required for centromere function. Nonetheless, neocentromeres display a premature centromere separation upon colcemid treatment, suggesting a defect in centromere cohesion that may be attributable to the absence of heterochromatin.
In a unique case of centromere repositioning, a neocentromere has formed in band 8q21.3 in an otherwise unrearranged chromosome with an inactivated endogenous centromere. CENP-A ChIP-seq has shown that this neocentromere is located on a large variable tandemly repeated DNA, unlike all other mapped neocentromeres which were found in single copy non-repetitive DNA. In control cell lines this repeat is largely covered with H3K9me3 heterochromatin. Upon neocentromere formation, this H3K9me3 is largely displaced, resulting in an alternating CENP-A and H3K9me3 pattern which may define a repeating subunit. This neocentromere contains heterochromatin, and notably, does not show the premature centromere separation observed at neocentromeres that lack heterochromatin. Thus, these studies have taken advantage of human neocentromeres to examine the epigenetic requirements for human centromere function.

MINIMAL FUNCTIONAL TELOMERE LENGTH IS CHROMOSOME ARM DEPENDENT.

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Telomeres are the nucleoprotein structures that protect the ends of eukaryotic chromosomes. Telomeres are maintained by the enzyme telomerase, and *Arabidopsis* plants deficient for the catalytic subunit TERT survive for up to 10 sexual generations before severe telomere dysfunction causes massive genomic instability that renders the plants completely sterile. In an attempt to complement the *tert* mutation, a construct overexpressing a full-length *TERT* cDNA (pCBK29) was transformed into 4th generation tert mutants. This construct was unable to fully restore telomerase activity as telomeres continued to shorten despite the presence of telomerase activity detectable by TRAP assay. However, plants containing pCBK29 continue to be viable into at least the 16th generation, and analysis of telomere length shows that most telomeres are stably maintained at an extremely short length of between 350 and 450 bp. We conclude that plants containing pCBK29 can not efficiently elongate fully functional telomeres but can maintain telomeres that are becoming deprotected and maintain them close to a minimal functional length. Surprisingly, two chromosome arms are maintained at a longer length of approximately 800 to 1200 bp. Compared to the other chromosome arms, these two arms have expressed genes located in extremely close proximity to the telomere. ChIP analysis demonstrates that the histones located at these two arms have modifications reminiscent of highly transcribed euchromatic regions, while the immediate subtelomeric regions of the other chromosome arms are more heterochromatic. These data suggest that the hypomorphic allele of TERT encoded by pCBK29 may be differentially recruited to telomeres based either on their chromatin structure or transcriptional status.

H2A.Z NUCLEOSOMES DOWNSTREAM OF ACTIVE GENE PROMOTERS ARE HOMOTYPIC.

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Nucleosomes that contain the histone variant H2A.Z are enriched at promoters and other regulatory elements in many organisms. A single octameric nucleosome can contain two H2A.Z histones (homotypic) or one H2A.Z and one canonical H2A (heterotypic). It remained to be determined where these homotypic and heterotypic nucleosomes localize throughout the genome. To resolve this, we generated high-resolution maps of homotypic and heterotypic Drosophila H2Av nucleosomes, where H2Av is the unique homolog of the two universal histone variants H2A.Z and H2A.X. We found that homotypic and heterotypic H2Av nucleosomes map indistinguishably throughout most of the genome. However, homotypic nucleosomes are distinctively enriched immediately downstream of active promoters, showing well-positioned phasing that dampens out over gene bodies. In contrast, heterotypic nucleosomes are relatively depleted in these same regions. As homotypic and heterotypic nucleosomes are structurally and biochemically distinct, our results suggest that replacing H2A with H2A.Z in heterotypic nucleosomes to make them homotypic is a general process that helps to maintain gene activity.

MRNA EXPORT FROM MAMMALIAN CELL NUCLEI IS DEPENDENT ON GANP

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Bulk nuclear export of mRNPs through nuclear pore complexes (NPCs) is mediated by NXF1. It binds mRNPs through adaptor proteins such as ALY and SR splicing factors and mediates translocation through the central NPC transport channel using transient interactions with FG-nucleoporins. Here, we show that mammalian cells require GANP (Germinal-centre Associated Nuclear Protein) for efficient mRNP nuclear export and for efficient recruitment of NXF1 to NPCs. Separate regions of GANP show local homology to FG-nucleoporins, the yeast mRNA export factor Sac3p, and the mammalian MCM3 acetyltransferase. GANP interacts with both NXF1 and NPCs, and partitions between NPCs and the nuclear interior. GANP depletion inhibits mRNA export, with retention of mRNPs and NXF1 in punctate foci within the nucleus. The GANP N-terminal region that contains FG motifs interacts with the NXF1 FG-binding domain. Over-expression of this GANP fragment leads to nuclear accumulation of both poly(A)+RNA and NXF1. Treatment with transcription inhibitors redistributes GANP from NPCs into foci throughout the nucleus. These results establish GANP as an integral component of the mammalian mRNA export machinery and suggest a model whereby GANP facilitates the transfer of NXF1-containing mRNPs to NPCs[1]. We are currently investigating whether GANP is required for export of a subset of mRNA.

[1]Wickramasinghe, VO*., et al. mRNA export from mammalian cell nuclei is dependent on GANP. (2010) Curr Biol; 20:25-31 *Corresponding author

SINGLE MOLECULE IMAGING OF NATIVE CHROMATIN DURING CANCER PROGRESSION

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The prevalence of epigenetic alterations during cancer progression is widespread. Genome wide maps of locations of various histone modifications and variants have allowed us to infer much about their function based on their distribution. However, it must be kept in mind that these methods involve averages across populations and do not indicate what goes on at a single molecule level. Therefore, new applications of recent technologies are needed. Assays such as atomic force microscopy (AFM) have provided exceptional insight into biological processes at single molecule resolution. We have begun to use AFM to study changes in native chromatin during cancer progression by utilizing DNA aptamers, which allow for the highly specific recognition imaging of histone variants and post-translational modifications at the single molecule level. We will determine the variant and modification features of nucleosomes and native chromatin arrays in colon adenoma and adenocarcinoma cell lines by AFM-recognition imaging and genome-wide profiling.
Methylated DNA from a pair of human colon adenoma (AA/C1) and adenocarcinoma (AA/C1/SB10) cell lines was enriched using a MeDIP 5-methylcytosine immunoprecipitation and profiled using NimbleGen tiling arrays. Ends analysis reveals a slight increase in DNA methylation at gene promoters and a slight decrease in DNA methylation in gene bodies during cancer progression. We are currently profiling the occupancy of H2A.Z in these cell lines and performing expression microarrays. Therefore, we will be able to gain a comprehensive view of the changes that occur in gene expression, DNA methylation, and H2A.Z occupancy during progression from colon adenoma to adenocarcinoma. In addition, we have extracted native chromatin from the colon adenocarcinoma cell line and performed imaging at the single molecule resolution using atomic force microscopy (AFM). In low salt, individual nucleosomes are clearly resolved. We have also performed recognition imaging of native chromatin with a DNA aptamer to histone H4. This is the first use of the histone H4 aptamer on native human chromatin. We are currently developing DNA aptamers for the recognition of both H2A and H2A.Z. In addition, we have begun synthesis of a new three-way linker that will allow us to determine the co-localization of histone variants or modifications in a single recognition image. A greater understanding of the how epigenetic regulation becomes altered during tumor progression is necessary to provide new targets for epigenetic therapy.

A REGION OF THE HUMAN HOX D CLUSTER THAT CONFERS POLYCOMB-GROUP RESPONSIVENESS

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Cell fate decisions during embryonic development are maintained epigenetically by chromatin modifying proteins, such as the Polycomb group (PcG) proteins, and are essential for correct axial body patterning. In Drosophila, the PcG proteins are targeted to Polycomb response elements (PREs) to mediate the maintenance of gene silencing, most notably at the homeotic cluster. Despite characterization of PREs in Drosophila, very little is known about how PREs might function in higher vertebrates.

We have identified an intergenic region within the HOX D cluster that has the ability to repress gene expression in a Polycomb-dependent manner in differentiating human embryonic stem (ES) cells. Analyzing chromatin architecture in the context of a step-wise model of differentiation, we discovered a region between HOXD11 and D12 (D11.12) that was associated with PcG proteins (i.e. BMI1, SUZ12, and YY1) and was MNase hypersensitive. When tested in an in vitro assay, D11.12 was able to repress luciferase expression and moreover was dependent on BMI1 and EED. In addition, full repression by D11.12 required YY1 binding sites and a highly conserved region. Taken together, our results suggest that D11.12 is capable of repressing transcription in differentiating human ES cells and this functions in a Polycomb-dependent manner.

HERITABLE GENE DISRUPTION USING ZINC FINGER NUCLEASES REVEALS EVOLUTIONARY CONSERVATION OF DOSAGE COMPENSATION MACHINERY AMONG CAENORHABDITIS SPECIES

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The nematode *Caenorhabditis elegans* is a robust model organism for diverse biological studies, primarily due to its transparency and facile forward genetic screening approaches. However, reverse genetic techniques such as gene targeting are less well established in this system, but are nonetheless highly desirable tools for dissecting gene function. To this end, we have employed zinc finger nucleases (ZFNs) to achieve heritable, single-step gene targeting. ZFNs are proteins consisting of custom-designed DNA binding motifs based on the C2H2 zinc finger as a scaffold, fused to the cleavage domain of the *Fok*I restriction enzyme. These molecules have recently been applied to other model systems to generate double-strand breaks at pre-determined loci, which are then subject to imperfect repair via the non-homologous end-joining pathway to generate short insertions and deletions at the target site. To demonstrate proof of principle, mRNAs encoding ZFNs designed to target a GFP transgene were injected into the hermaphrodite gonad, yielding non-green progeny carrying a range of mutations at the target site. Evolutionary studies within the nematode phylum have been hampered by the lack of efficient gene targeting technologies outside of *Caenorhabditis elegans*. To address this issue, and to demonstrate the versatility of ZFN technology, we have studied the evolution of X chromosome dosage compensation in sister species of the *Caenorhabditis* clade. The *sdc-2* gene in *Caenorhabditis elegans* encodes the primary hermaphrodite determinant which also triggers loading of the dosage compensation complex onto the X chromosome. Through ZFN-driven targeted disruption of *sdc-2* in the related nematode *Caenorhabditis briggsae*, we find key features of the genetic hierarchy that equalizes X chromosome gene expression between the sexes to be conserved. Protocols are under development to allow the isolation of mutations and homology-directed replacements within any gene, regardless of the resulting phenotype. The successful application of ZFN technology to different species of *Caenorhabditis* adds a new dimension to the reverse genetic toolkit for these important model organisms.

CHROMATIN-INDUCED MICROTUBULE DEPOLYMERIZATION IS REQUIRED FOR NUCLEAR ASSEMBLY

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Spatial and temporal signals from chromatin coordinate the activities of many macromolecular assemblies in the cell. At exit from M phase, these signals switch from driving spindle microtubule assembly to stimulating nuclear envelope reformation. Here we report a novel chromosome-binding protein, Vespera, which couples these activities in *Xenopus* egg extracts and bears homology to mammalian Dppa2 and Dppa4. Vespera negatively regulates microtubule polymerization, and is essential for nuclear assembly. We found that Vespera is conjugated to the small ubiquitin-like modifier (SUMO) by the SUMO E3 ligase PIASy upon binding to chromatin, and this modification was essential for its activity. Addition of excess Vespera prevented assembly of metaphase spindle microtubules, while depletion of Vespera inhibited postmitotic nuclear assembly, resulting in pinched and deformed nuclear shapes. Strikingly, the effects of Vespera depletion were rescued by the microtubule-depolymerizing agent nocodazole, which restored normal, round nuclei with full nuclear import function. Conversely, the microtubule-stabilizing drug taxol inhibited nuclear assembly in undepleted extracts. These results suggest, therefore, that microtubule depolymerization at mitotic exit by SUMOylated Vespera is necessary for subsequent nuclear assembly. Moreover, we found that Vespera functionally opposes the kinase Aurora B, which phosphorylates many substrates to promote spindle assembly and must be removed from chromatin for nuclear reformation. We found that co-depletion of Aurora B rescued the nuclear assembly defects of Vespera depletion, while removal of Vespera delayed the dephosphorylation of Aurora B substrates. The activities of both Vespera and Aurora B are stimulated by chromatin, and thus their mutual antagonism may act as part of a sophisticated signaling network, in which posttranslational modifications such as SUMOylation contribute to the dynamic control of major cell cycle transitions.

COMPARATIVE ANALYSIS OF DNA REPLICATION TIMING REVEALS CONSERVED LARGE-SCALE CHROMOSOMAL ARCHITECTURE

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Recent evidence suggests that the timing of DNA replication is coordinated across megabase-scale domains in metazoan genomes, yet the importance of this aspect of genome organization is still unclear.
Here we show that replication timing is remarkably conserved between human and mouse, uncovering large regions that may have been governed by similar replication dynamics since these species have diverged.
This conservation is both tissue specific and independent of the genomic G+C content conservation. Moreover, we show that time of replication is globally conserved around sites of large scale genome rearrangements, but that at local fusion points early replicating domains preferentially invade late replicating domains. Rearrangement are shown to be correlated with early replication and physical chromosomal proximity. These results suggest that large chromosomal domains of coordinated replication are shuffled by evolution while conserving the large scale nuclear architecture of the genome.

MECHANISTIC STUDIES OF RNA POLYMERASE II SPECIES SPECIFIC TRANSCRIPTION INITIATION PATTERNS

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The basal eukaryotic transcription machinery for protein coding genes is highly conserved from yeast to human cells. However, while human cells usually initiate at a single transcription start site approximately 30 bp downstream of the TATA element, Schizosaccharomyces pombe typically initiates at multiple sites 30-70 bp, and Saccharomyces cerevisiae 40 to 200 bp downstream of TATA. The determinant factor(s) for the species specific initiation and the underlying mechanism(s) for the multiple downstream start site utilization in yeasts are not fully understood.

By swapping highly purified transcription factors between S. pombe and S. cerevisiae reconstituted transcription systems, we confirmed previous observations that RNA polymerase II and the general transcription factor TFIIB determine the species specific start site utilization. Further genetic and biochemical assays of TFIIB chimeras indicated that neither the N-terminal RNAPII binding domain nor the B-finger of TFIIB determines the species specific initiation patterns. Results from our ongoing studies analyzing the role of the TFIIB C-terminal domain in species specific start site utilization will also be presented. Bubble template initiation assays showed that there is a negative correlation between the amount of negative charge in the TFIIB B-finger tip and the efficiency of early phosphodiester bonds formation. Moreover, TFIIB mutants conferring further downstream start sites shift in S. cerevisiae showed no defect in early phosphodiester bond formation, suggesting that subsequent step(s) in the transcription cycle contribute to further downstream start site utilization.

GENOME SEQUENCING OF TWO SOCIALLY DISTINCT ANT SPECIES: *CAMPONOTUS FLORIDANUS* AND *HARPEGNATHOS SALTATOR*

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Next-Gen sequencing technology has enabled efficient genome mapping and assembly. Here we present the whole genome drafts of two ant species: *Camponotus floridanus* and *Harpegnathos saltator*. These two species represent distinct social structure and organization, and provide excellent models to study neurobiology, aging and development in a social colony environment. We compared candidate gene expression levels related to aging and development among the castes, and are investigating the epigenetic mechanisms controlling such differentiation. These research will facilitate the study of the complex eusocial insect in the laboratory system and further our understanding of the intricate epigenetic control behind social insect development.

DNA ZIP CODES: AN ANCIENT MECHANISM FOR GENE TARGETING TO THE NUCLEAR PERIPHERY.

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The subnuclear localization of DNA has important roles in regulation of transcription. A number of highly expressed genes are dynamically recruited to the nuclear periphery upon transcriptional activation in yeast. We have found that peripheral relocalization of INO1 is controlled by cis acting elements; when INO1 and flanking sequences were integrated at a distant locus in the genome, this locus relocalized to the nuclear periphery upon INO1 activation. We have identified an 8bp sequence that is necessary and sufficient for gene recruitment. Point mutations in this gene recruitment sequence (GRS) that block targeting of INO1 to the nuclear periphery also resulted in a defect in transcriptional activation, suggesting that peripheral localization promotes transcription. We have found that a GRS element in the promoter of the unrelated TSA2 gene was also necessary for TSA2 targeting to the nuclear periphery suggesting that GRS mediated targeting is a general mechanism of targeting for other yeast genes. Targeting of either INO1 or the GRS alone was blocked in mutants lacking a number of components of the nuclear pore complex and associated factors. Finally, the GRS was sufficient to target DNA to the nuclear periphery in the distantly related yeast species *Schizosaccharomyces pombe*, suggesting that this targeting mechanism is conserved over at least one billion years of evolution.

NUCLEAR DEADENYLATION, POLYADENYLATION AND TUMOR SUPPRESSOR FACTORS REGULATE 3' PROCESSING IN RESPONSE TO DNA DAMAGE.

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RNA 3’ end processing is strongly but transiently inhibited upon DNA damaging conditions. Initially, it was described that this process is inhibited after UV-induced DNA damage as a result of both the formation of the BARD1/CstF complex and the proteasomal-mediated degradation of RNA polymerase II (RNAP II). As CstF-50 can interact with the tumor suppressor BARD1 to inhibit and with RNAP II to activate 3’ processing, it was proposed that CstF-50 plays a coordinating role in the nuclear response to DNA damage. Supporting this idea, cells with reduced levels of CstF show both an enhanced sensitivity to UV and reduced ability to ubiquitinate RNAP II and repair DNA. To further understand the role of CstF-50 in the DNA damage response, we study other CstF-50 interactors. CstF-50 also interacts with the poly(A)-specific ribonuclease (PARN) upon UV treatment. Based on the nature of the factors, it is possible to hypothesize that the PARN/CstF-50 interaction might regulate mRNA turnover in different cellular responses. Consistent with this, the CstF-50/PARN complex plays a role in inhibition of 3’ cleavage and activation of deadenylation upon DNA damage. Here we show that the UV-induced activation of deadenylation in NEs was enhanced by the presence of m7-guanosine cap on the substrate RNA, supporting previous observations showing that PARN activity is stimulated by 5’ cap. We show that BARD1 plays an important role in the activation of PARN-mediated deadenylation after UV treatment through its interaction with CstF. BARD1/CstF-50 complex strongly activates deadenylation by PARN and can revert the CBP80-mediated inhibition of PARN activity. We determined that not only PARN expression but also the BARD1/CstF/PARN complex formation play an important role in regulating mRNA levels of housekeeping genes upon DNA damaging conditions, and thereby might contribute to the UV-induced decrease in the cellular levels of total mRNA. Then we extended these studies to short lived mRNAs. Strikingly, PARN knockdown cells showed increase in the stability of both c-fos and c-myc in samples from non-UV treated cells. These results suggest that PARN plays a role decreasing the stability of short lived mRNAs, keeping their expression levels low in non-stress conditions. The reduced expression of PARN has a slight effect on the UV-induced increase in the expression levels of these genes, suggesting that other mechanism(s) might be involved in determining the stability of these genes upon DNA damage conditions.

COMPARATIVE VISUALIZATION OF TRANSCRIPTIONAL INDUCTION IN INTERPHASE AND POST-MITOTIC CELLS

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A comparative analysis of the kinetics of transcriptional induction of the same genetic locus in interphase and post-mitotic cells was performed using 4D-microscopy. In interphase, after transcriptional induction, a gradual accumulation of RNA polymerase II large sub-unit (RNAPIILS) and nascent transcripts was observed at the locus, taking ~180 minutes to reach their maximal signal intensity. Next, we kept the locus in the transcriptionally active state and allowed it to naturally shut-down upon entry into mitosis. Upon exit from mitosis, RNAPIILS was recruited to the locus in telophase and reached its maximal signal intensity in 2-4 min, with mRNA production peaking within 15-30 min. This observation suggested that the cell is able to remember, or "bookmark", the locus as one needing to be transcribed such that the transcription machinery can be rapidly recruited to the locus when cellular transcription is reactivated upon exit from mitosis. Interestingly, we also detect a rapid recruitment of Brd4 in early G1, which occurs ~2-4 mins earlier than RNAPIILS and positive transcription elongation factor b (P-TEFb). Chromosome immunoprecipitation (ChIP) experiments showed ~4 fold enrichment of Histone H4 lysine 5 Acetylation (H4K5Ac) at the locus upon induction in interphase. These data suggest that both Brd4 and the histone modification pattern are part of a "bookmarking" complex at the locus. Interestingly, when transcription elongation is inhibited prior to mitosis by treating cells with 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) and then releasing them to allow the cells to proceed though mitosis, RNAPIILS is still rapidly recruited to the locus, although mRNA synthesis initiates more slowly. These results imply that transcriptional initiation and transcriptional elongation are separately bookmarked. Our study provides a real-time view of interphase transcriptional induction and post-mitotic reactivation at a single genetic locus and provides a system in which to study how gene expression patterns are transmitted to daughter cells.

SUMO MODIFICATION OF BLM REGULATES ITS INTERACTION WITH RAD51 AT DAMAGED DNA REPLICATION FORKS AND AFFECTS RNF4-MEDIATED UBIQUITINATION

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Bloom's syndrome (BS) is an autosomal recessive human genetic disease caused by mutations in the gene encoding for BLM, a protein belonging to the RecQ family of DNA helicases. We previously demonstrated that BLM is modified by the small ubiquitin-related modifier (SUMO) and that sumoylation plays an important role in regulating its trafficking between PML nuclear bodies and DNA repair foci. To further understand the effects of sumoylation on BLM's DNA repair functions, we characterized cells expressing a sumoylation deficient BLM mutant protein (SM-BLM) and found that they are hypersensitive to DNA damage reagents and that they are defective in homologous recombination-mediated DNA repair. As RAD51 is the key recombinase involved in HR, we hypothesized that the function of RAD51 could be compromised. Consistent with this hypothesis, we found that the localization of RAD51 to DNA repair foci was impaired in SM-BLM cells. Further studies demonstrated that RAD51 interacts noncovalently with SUMO in vitro, and it interacts more efficiently with SUMO-modified BLM compared to unmodified BLM. We also found that SUMO modified BLM could be recognized and ubiquitinated by the SUMO-dependent ubiquitin E3 ligase, RNF4. DNA damage, RNF4 depletion, or proteasome inhibition led to the accumulation of poly-sumoylated BLM. Based on these findings, we propose that sumoylation of BLM functions to both facilitate the recruitment of RAD51 to stalled DNA replication forks and to mediate the turnover of BLM upon DNA damage repair.

DYNAMICS OF GENOME – NUCLEAR LAMINA INTERACTIONS.

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The nuclear lamina is thought to provide an important anchoring scaffold for the organization of interphase chromosomes, and may also play an active role in the regulation of gene expression. We present a high resolution genome-wide analysis of the interactions between chromatin and the nuclear lamina during differentiation. We mapped these interactions in mouse embryonic stem cells, lineage-committed neural precursor cells, and terminally differentiated astrocytes that are sequentially derived from one another.

Chromatin in each of these cell types shows a similar organization into large lamina associated domains (LADs). The overall similarity of lamina interaction patterns between the very different cell types points to a common "core architecture" of interphase chromosomes. During sequential differentiation steps, this core architecture is progressively modified at hundreds of genomic locations. This remodeling of lamina interactions involves both individual transcription units and multi-gene regions, and affects many genes that determine cellular identity. Often, genes that move away from the lamina are concomitantly activated; many others however remain inactive yet become unlocked for activation in a next differentiation step.

These results suggest that lamina-genome interactions are widely involved in the control of gene expression programs during lineage commitment and terminal differentiation.

THE NUCLEAR LAMINS: BUILDING BLOCKS OF NUCLEAR ARCHITECTURE

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The nuclear lamins are Type V intermediate filament (IF) proteins that are encoded by two B-type lamin genes and one A-type lamin gene. These IF proteins polymerize into separate A- and B-type nuclear networks that are located in the nuclear lamina and elsewhere in the nucleoplasm. The lamins are involved in many functions including DNA replication, mitosis, transcription, the organization of interphase chromosome territories and in the overall shape and mechanical properties of nuclei. Remarkably, approximately 340 mutations have been reported in the human nuclear lamin A gene. These mutations cause many different diseases, including muscular dystrophies, cardiomyopathies, lipodystrophies, and Hutchinson-Gilford Progeria Syndrome (HGPS), an early onset premature aging disease. These diseases are frequently accompanied by the formation of blebbed or lobulated nuclei, the mislocalization of nuclear pores and membrane proteins, and significant alterations in the epigenetic regulation and organization of interphase chromosomes. Since little is known about the specific cellular and molecular mechanisms altered by the expression of these lamin A mutations, we have initiated studies to determine the impact of the mutant proteins on normal nuclear functions. Most of our studies have focused on the mutations that cause HGPS or other atypical forms of progeria. One of the hallmarks of cells derived from progeria patients is their premature senescence, a property that is most likely to be directly relevant to patients with these diseases. In light of this we have initiated studies to determine the roles of both mutant and wild type lamins in cellular senescence. The data obtained to date are shedding new light on normal lamin functions and the alterations in these functions when mutant lamin As are expressed. Our results have led us to hypothesize that lamins form a nuclear scaffolding complex that provides a molecular interface between chromatin and the lamina/nuclear envelope and between chromatin and other components of the nucleoplasm. This lamin scaffold also acts as a platform for the assembly of protein complexes involved in a wide range of functions. Supported by the NIA and the NCI .

REGULATION AND FUNCTIONS OF NUCLEOPLASMIC LAMINS IN TISSUE PROGENITOR CELLS

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Lamins are nuclear intermediate filament proteins in metazoans that form a scaffolding network at the nuclear envelope, called the lamina. While B-type lamins are ubiquitously expressed, A-type lamins are expressed at later stages of development, and mutations in the LMNA gene cause various pathologies ranging from striated muscle defects to premature ageing syndromes. It has been suggested that disease-specific defects of the peripheral lamina affecting nuclear architecture, gene expression and signaling may cause the pathologies. We and others have recently introduced a new model showing that, in addition to the major lamin structures at the nuclear periphery, a small pool of lamin A/C locates in the nuclear interior and is involved in retinoblastoma-mediated gene expression control. Interestingly, nucleoplasmic lamins are found only in G1 phase of proliferating cells in culture and in vivo in regenerating tissue, while they are lost during S-phase and in non-proliferating, differentiated cells. The regulation and functions of this lamin pool are poorly understood. We show that nucleoplasmic targeting of lamin A/C requires a nucleoplasmic lamin-binding protein, termed lamin-associated polypeptide 2 alpha (LAP2α), which belongs to the LEM protein family known to interact with chromatin. LAP2α downregulation in cells causes defects in cell cycle withdrawal following serum starvation or contact inhibition, and loss of LAP2α in mice induces hyperproliferation of early tissue progenitor cells in epidermal, striated muscle- and hematopoietic tissues. Interestingly, these cellular phenotypes are accompanied by loss of intranuclear lamin A and are rescued by re-expression of LAP2α and restoration of the nucleoplasmic lamin A pool. Furthermore, the observed cell cycle phenotype caused by loss of LAP2α function requires a functional retinoblastoma pathway. Our data show that complexes of LAP2α and A-type lamins in the nuclear interior regulate the balance between proliferation and differentiation of early progenitor cells in regenerating tissues in a retinoblastoma-dependent manner. We propose that disease-causing mutations in lamins may alter the nucleoplasmic pool of lamin A/C and thereby disturb tissue homeostasis in patients. This study was supported by the Austrian Science Research Fund and the EURO-Laminopathies research project of the European Commission (Contract LSHM-CT-2005-018690).

DIFFERENTIATION SPECIFIC POSITIONING OF TISSUE-SPECIFIC GENES DURING *C. ELEGANS* DEVELOPMENT IS PERTURBED BY A DISEASE-CAUSING LAMIN MUTATION

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Chromatin is non-randomly organized in the interphase nucleus. In particular, studies with mammalian cell culture systems revealed that silent loci often localize to the nuclear periphery where they interact with the nuclear lamina. While the clustering of repeat sequences in yeast facilitates SIR-mediated silencing, it is unclear whether clustering of silent genes at the nuclear envelope in higher eukaryotes has any biological function. One possibility is that this would facilitate heritable maintenance of a repressed state by creating a zone within which de novo assembled chromatin after replication would preferentially be silenced. In order to study the subnuclear distribution of genes in vivo and throughout development we have used recognition of lacO repeats by GFP-lacI for visualization of genetic loci in *C. elegans*.
Low-copy integrated transgenes that contain arrays of LacO sites, and developmentally regulated promoters (*myo-3*::mCherry or *pha-4*::mCherry-H2B), are used to analyse cell-type specific nuclear organization during development. In early embryos, transgenes bearing inactive tissue-specific promoters are randomly distributed throughout nuclear space. Over the course of differentiation, we observe spatial segregation of the transgenes, depending on their transcriptional status. Inactive silent transgenes shift to be highly enriched at the nuclear periphery while active promoters are sequestered internally. This is true for at least four tissues.
As opposed to low-copy transgenes obtained by bombardment, integrated repetitive transgene arrays derived form gonadal plasmid injection accumulate massive amounts of heterochromatic marks (H3K9me3 and H3K27me3). These silent domains are attached to the nuclear periphery by default throughout development. However, similar to low-copy transgenes, repetitive arrays can overcome peripheral anchoring upon activation of a developmentally regulated promoter located on them.
Lamin mutations alter the spatial organization of heterochromatic arrays, and one specific mutation, which confers Emery Dreifuss muscular dystrophy in humans, specifically interferes with the subnuclear repositioning of a muscle-specific promoter array. This mutation further interferes with the induction of array-borne myo-3 promoters in muscle cells. We conclude that nuclear organization is cell type specific and helps maintain transcriptional patterns in differentiated cells.

THE ARCHITECTURE AND TRANSPORT MECHANISM OF THE NUCLEAR PORE COMPLEX

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Nucleocytoplasmic transport occurs through nuclear pore complexes (NPCs), macromolecular structures embedded within the nuclear envelope. Composed of nucleoporins (nups), NPCs mediate bi-directional trafficking between the nucleoplasm and cytoplasm, acting as a dynamic barrier to control access to the nucleus. We have previously determined the position of every nup with a precision of ~6 nm. At this resolution, we see an arrangement of coaxial rings forming an elaborate scaffold that defines a ~30 nm diameter passageway between the nucleus and cytoplasm. We have also assigned fold types to ~98% of the nups, exposing a modularity in the architecture of the NPC; observed similarities between structures in coated vesicles and those in the NPC support our hypothesis for their common evolutionary origin in a progenitor protocoatomer. In order to understand at higher resolution how the NPC is assembled, we are now mapping the morphology and connectivity of the nup complexes constituting the NPC. The scaffold of the NPC also anchors proteins termed FG-nups, whose natively disordered domains line the central passageway. While FG nups form an effective barrier to the diffusion of most macromolecules across the NPC, cargo-carrying transport factors overcome this barrier by transient binding to the FG-nups. To test whether nothing more than a passageway and a lining of FG-nups are sufficient for selective transport, we designed functionalized membranes that incorporate just these two elements. We show that these membranes function as nanoselective filters, efficiently passing transport factors and transport factor-cargo complexes that specifically bind FG-nups, whilst significantly inhibiting the passage of proteins that do not bind. Using this system as well as other approaches, we have gained several key insights into the factors governing the selectivity and efficiency of nuclear transport.

CELL CYCLE DEPENDENT DIFFERENCES IN NUCLEAR PORE COMPLEX ASSEMBLY IN METAZOA

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Nuclear pore complexes (NPCs) are the exclusive channels of nucleo-cytoplasmic transport in eukaryotic cells. These multiprotein assemblies have an estimated mass of ~60 MD and are embedded within the double lipid bilayer of the nuclear envelope (NE). Each NPC assembles from ~30 different nucleoporins (Nups), present in multiple copies, amounting to a total of ~500 polypeptides. NPCs consist of a NE-embedded scaffold surrounding the central channel, largely composed of the Nup107/160 and Nup93/Nup205 complexes. The Nup107/160 complex has been shown to be an early and essential player in NPC formation both in vitro and in vivo.

In metazoa, NPCs assemble from disassembled precursors into a reforming NE at the end of mitosis, and into growing intact NEs during interphase. We show that the RNAi-mediated knockdown of ELYS, a nucleoporin critical for the recruitment of the essential Nup107/160 complex to chromatin, blocks NPC assembly at the end of mitosis, but not during interphase. Conversely, the transmembrane nucleoporin POM121 is critical for the incorporation of the Nup107/160 complex into new assembly sites specifically during interphase. Strikingly, the recruitment of the Nup107/160 complex to an intact NE involves a membrane curvature-sensing domain of its constituent Nup133, which is not required in post-mitotic NPC formation. Our results suggest that, in organisms with open mitosis, NPCs assemble by two distinct mechanisms to accommodate cell cycle-dependent differences in NE topology.

CHROMATIN HIGHER ORDER STRUCTURE AND REGULATION OF ITS COMPACTION

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During the past decade it has become evident that histone post-translational modifications are key regulators of all nuclear processes whose substrate is DNA. Whilst the effects of, for instance, histone post-translational modification on transcription are well-documented, there is no mechanistic understanding of how such modification regulate chromatin condensation directly, or indirectly. Such an understanding is dependent on knowledge of the three-dimensional structure of chromatin. Although the structure of the first level of DNA folding, the nucleosome core, is known at atomic resolution, the structure of the second level of folding, whereby a string of nucleosomes folds into a fibre with an approximate diameter of 30 nm - the “30 nm” chromatin fibre, remains undetermined. I will describe our studies on the higher orders structure of chromatin with two primary aims:
1) Determination of the structure of the “30nm’ chromatin fibre to provide an understanding of fibre topology.
2) Biophysical characterization of the effects of the linker histone and histone modifications on the compaction of chromatin higher order structure.

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INSIGHTS INTO INTERPHASE LARGE-SCALE CHROMATIN STRUCTURE FROM ANALYSIS OF ENGINEERED CHROMOSOME REGIONS

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How 10 and 30 nm chromatin fibers fold into mitotic and interphase chromosomes has remained a difficult question for many years. This has been as much a problem of experimental methods for visualizing specific sequences as a problem of limitations of resolution and spatial dynamic range of available microscopy methods. Over a number of years we have used engineered chromosome regions tagged with lac operator repeats as a means of visualizing the structure of specific chromosome regions in live cells and cells fixed under conditions which preserve large-scale chromatin structure while also facilitating a reductionist experimental approach. We have progressed from gene amplified chromosome regions to regions created by multi-copy integration of plasmids, and now to engineered chromosome regions formed by multi-copy BAC repeats carrying 100-200 kb of defined genomic loci. We also recently have developed a novel in vivo immunogold labeling procedure to visualize these chromosome regions at the ultrastructural level without perturbation induced by exposure to detergents and buffer conditions which might alter chromatin conformation.

Here we summarize insights into interphase and mitotic large-scale chromatin folding and dynamics using these engineered chromosome regions. Our recent results with the multi-copy BAC constructs suggest that transcription at levels approaching within several fold the level of the corresponding endogenous gene locus occurs on a condensed template which, however, undergoes a global, long-range several fold decondensation with transcriptional induction. Experiments examining engineered chromosome regions in mouse ES cells suggest a surprising degree of plasticity and self-organization in large-scale chromatin structure during differentiation.

We have begun to apply these engineered chromosome regions to determine cis and trans determinants of large-scale chromatin structure.

CHROMOSOME TERRITORIES AND THE INTERCHROMATIN COMPARTMENT: A FUNCTIONAL MARRIAGE.

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Progress made during the last few years has underlined the functional importance of the space-time dynamics of higher order chromatin organization in cycling and postmitotic cells. Whereas chromosome territories (CTs) are generally accepted as a basic feature of higher order chromatin organization, the dynamic structure and topography of chromatin domains and loops is still not well understood. The large gap, which must still be bridged from the molecular level to the level of higher-order nuclear organization, is emphasized by currently discussed models of nuclear architecture. The CT-IC model predicts an interchromatin compartment (IC), which harbors splicing speckles and nuclear bodies, is largely devoid of DNA and pervades the nucleus, in particular its ‘euchromatic’ regions, between and within CTs (1,2). The interior of the IC is separated from the compacted interior of chromatin domains by a zone of decondensed chromatin, called the perichromatin region (PR). State-of-the-art light and electron microscopic methods provide evidence that the PR serves as the functional subcompartment for transcription, co-transcriptional splicing, DNA-replication and possibly also DNA repair (3).

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THE MODULAR STRUCTURE OF CHROMOSOMAL NETWORKS

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Work during the last few years has indicated that direct physical interactions between chromosomes act as novel regulators of the expressivity of the genome. Thus, distant interactions among chromosomal loci are increasingly being seen as an important third dimension of genome biology (1). While it is generally assumed that regulatory elements from neighbouring domains or from other chromosomes interact in association to transcription or repression, this may be a too simplistic view. Using the 4C approach we have discovered the "imprintome", a network of physically interacting transcriptional units with the common denominator of being genomically imprinted (2,3). These features are linked with transvection of epigenetic states, i.e. DNA methylation (4) and replication timing patterns (3), although the involved causal relationships are currently not well understood. This may relate to the possibility that chromosomal networks have a dual relationship to epigenetic states, i.e. epigenetic marks are not only likely directly or indirectly responsible for the initial contacts between chromosomes, but can also be transferred between chromatin fibres to provide examples of feed-forward behaviour of chromosomal networks (2-4). Thus, while it is known that the genome extensively interacts with itself it is not known how these interactions are organized and controlled. Using our 4C technique, which has the ability to capture several, simultaneously occuring interactions (2), we have identified a network with at least three simultaneously interacting sequences. As many of these interacted with each other, we have been able to generate an interactome displaying multiple nodes. Power-Law analysis reveals that this chromosome interactome is scale-free in human embryoid bodies. This information shows that there are nodes with a high degree of connectivity to other chromosomes suggesting that chromosomal networks are hierarchially structured with connectors and outliers. We will discuss the possibility that these nodes are organized in modules with functional crosstalk.

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THE TRANSCRIPTIONAL INTERACTOME, PREFERENTIAL SPATIAL ORGANIZATION OF CO-REGULATED GENES

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The discovery of interchromosomal interactions in higher eukaryotes suggests a functional link between nuclear genome organization and gene expression. We find that transcripitonally active genes share RNA polymerase II enriched nuclear foci known as transcription factories, challenging the view of transcription as a one-dimensional process. The extent of intra- and inter-chromosomal sharing of factories and the underlying mechanisms are unknown. Here we present a genome-wide analysis of genes that share transcription factories with the mouse alpha- and beta-globin genes (Hba & Hbb) in erythroid tissues. Our results show that the active globin genes associate with hundreds of other transcribed genes, revealing extensive and preferential intra- and interchromosomal transcription interactomes. Transcription units sharing transcription factories with the globin genes are overrepresented in genes regulated by the erythroid-specific Kruppel-like transcription factor, Klf1. We show that Klf1 mediates preferential co-associations of multiple Klf1-regulated genes at a limited number of specialized transcription factories that contain high levels of Klf1. Our results establish a new gene expression paradigm, suggesting that active co-regulated genes and their regulatory factors cooperate to create specialized nuclear hotspots optimized for efficient and coordinated transcriptional control.

NUCLEAR ORGANIZATION AND DYNAMICS OF THE H3K27 DEMETHYLASE JMJD3

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We have been interested in the functional organization of jumanji (Jmj) proteins in the mammalian cell nucleus. A group of H3K27 demethylases has been identified which can impact the H3K27 methylation status of genes that are activated upon cellular differentiation or exogenous signals. We have performed live cell imaging of the H3K27 demethylase JMJD3 to elucidate its localization and dynamics in human cells. In addition to a diffuse nuclear pool, endogenous or YFP-tagged JMJD3 protein localizes to 20-40 nuclear foci, which are 0.2-0.5 μm in diameter. In interphase, JMJD3 foci co-localize with heterochromatin rich in H3K9me3, HP1α, HP1β and HP1γ. During mitosis, the foci disassemble in prometaphase, and they are formed once again in early G1. A mutational and deletion analysis indicates that the localization of JMJD3 in nuclear foci depends on an intact N-terminus and Zn-finger domain. The diffuse, and foci associated JMJD3 can be distinguished by their mobility in a fluorescence recovery after photobleaching (FRAP) experiment. JMJD3 residing in foci has a significantly lower mobility than the diffuse pool, and the lower mobility depends on an intact JmjC domain. Interestingly, JMJD3 gets rapidly (within 3 min) and transiently recruited to a reporter gene upon transcriptional activation. Our data indicate a function of JMJD3 in transcriptional activation and/or chromatin remodeling. We are currently investigating the role of JMJD3 in transcriptional induction of endogenous genes.

RESETTING THE LINEAGE CLOCK – HOW DOES REPROGRAMMING WORK ?

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Differentiated uni-potent cells can be converted back to a multi-potent state by different experimental means - including nuclear transfer into enucleated oocytes, and the force expression of key factors to generate Induced Pluripotent Stem (IPS) cells.
The success rates for both these approaches are vanishingly low even after optimization (1-2%), which means that it is difficult to assess at the molecular level the important events that occur early in reprogramming and to discriminate those that are essential for productive versus non-productive lineage conversion.
We have taken a different approach, by forming transient heterokaryons between differentiated cells (such as lymphocytes or fibroblasts), and pluripotent stem cells. Under these conditions the nucleus of the differentiated cell is rapidly restructured and chromatin remodeled to resemble that of a pluripotent cell, prior to the initiation of a pluripotent gene expression program and silencing of differentiation-associated genes.
We have used this approach with conditional ES mutants and RNAi-based approaches, to directly dissect the factors and mechanisms required for lineage conversion, as well as to elaborate how cells normally retain lineage identity through division.

GENE REPRESSION VIA CHROMATIN COMPACTION INDEPENDENT OF HISTONE MODIFICATION

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Polycomb mediated repression is thought to be mediated via the formation of facultative heterochromatin, but exactly how polycomb group proteins repress gene expression in vivo is not known. Whilst histone modifying activities of the polycomb repressive complexes (PRCs) have been studied extensively, in vitro data has suggested a direct activity of the PRC1 complex in compacting chromatin. We have investigated the higher-order chromatin compaction of polycomb targets in the nucleus. We show that polycomb repressive complexes are required to maintain a compact chromatin state at Hox loci in embryonic stem (ES) cells. There is specific decompaction in the absence of PRC2 or PRC1. This is due to PRC1, since decompaction occurs in Ring1B null cells that still have PRC2-mediated H3K27 methylation. Moreover, we show that the ability of PRC1 to restore a compact chromatin state, and to repress Hox gene expression in ES cells, is not dependent on the histone ubiquitination activity of Ring1B. We suggest that PRC1-mediated chromatin compaction acts to directly limit transcription in vivo.

POLYCOMB PROTEINS AND NUCLEAR ORGANIZATION DURING FLY DEVELOPMENT

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Polycomb Group (PcG) and trithorax group (trxG) proteins form multimeric protein complexes that regulate chromatin via histone modifications and modulation of nucleosome remodeling activities, targeted to specific cis-regulatory elements named PcG response elements (PREs). Upon binding to chromatin, these proteins maintain the memory of regulatory states of crucial developmental target genes, such as Hox genes. However, they can also dynamically bind to other genes and affect cell proliferation and differentiation in a wide variety of biological processes.
In addition to silencing the genes flanking their genomic target sites, PcG proteins play a role in nuclear organization. Staining for these proteins shows a distribution in foci called Polycomb bodies. We found that Polycomb bodies are the physical sites of Polycomb-mediated silencing. Moreover, endogenous PcG target loci can frequently colocalize in the cell nucleus. This is reflected by molecular contacts detected by chromosome conformation capture. Polycomb-dependent contacts increase the stability of chromatin silencing, suggesting that they play an important developmental role.

LONG NONCODING RNAS OF THE X-INACTIVATION CENTER

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The X-linked region now known as the 'X-inactivation center' (Xic) was once dominated by protein-coding genes but, with the rise of Eutherian mammals some 150-200 million years ago, became infiltrated by genes that produce long noncoding RNA (ncRNA). Some of the noncoding genes have been shown to play crucial roles during X-chromosome inactivation (XCI), including the targeting of chromatin modifiers to the X. The rapid establishment of ncRNA hints at a possible preference for long transcripts in some aspects of epigenetic regulation. I will discuss the role of noncoding genes during XCI, particularly in chromosome pairing, 3D interactions, and the initiation of silencing, and consider advantages RNA offers in delivering allelic, cis-limited, and locus-specific control.

TRIPLEX FORMATION BETWEEN NONCODING RNA AND DNA TARGETS DNMT3B TO REGULATORY ELEMENTS

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Heterochromatin formation and epigenetic silencing of a subpopulation of rRNA genes (rDNA) requires ‘pRNA’, 150-250 nt RNA that originates from the intergenic spacer and is complementary to the rDNA promoter. pRNA targets the chromatin remodeling complex NoRC to rDNA which in turn triggers nucleosome re-positioning and heterochromatin formation. Overexpression of pRNA mediates de novo DNA methylation and transcriptional repression, whereas antisense-mediated depletion of pRNA leads to displacement of NoRC from nucleoli and decreased rDNA methylation. pRNA is composed of two functional domains, a hairpin structure that is bound by NoRC and is required for heterochromatic histone modifications, and a 5’-terminal region that triggers DNA methylation and gene silencing. A 20 nt sequence in the 5’-terminal part of pRNA forms a DNA:RNA triplex with the binding site of a transcription factor adjacent to the rDNA promoter, and this triplex structure is specifically recognized by the DNA methyltransferase DNMT3b. Consistently, ectopic cyclin D1-specific ncRNA capable of triplex formation enhanced DNMT3b occupancy and hypermethylation of the cyclin D1 promoter. The results reveal a compelling new mechanism of RNA-dependent DNA methylation, suggesting that triplex-mediated targeting of DNMT3b to specific gene sequences is a common and widely used pathway in epigenetic regulation.

CHROMATIN DOMAINS AND NUCLEAR POSITIONING OF DNA HYPERMETHYLATED AND ABNORMALLY SILENCED GENES IN CANCER

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Aberrant promoter, CpG island DNA-hypermethylation and associated repressive chromatin constitutes a frequent mechanism of gene inactivation in cancer. We have been addressing the mechanisms underlying this abnormal silencing and have recently turned our attention to the role of gene chromatin domains and nuclear positioning in rendering genes vulnerable to this cancer-specific change. In terms of the former, we have associated a history of polycomb (PcG) marking in embryonic stem/progenitor cells with a large percentage of genes which adopt the above abnormal promoter DNA hypermethylation in duman colon and other cancers. In colon cancer cells, this PcG marking is in the context of "bivalent chromatin" which can be best visualized in the setting of manipulations which diminish the promoter DNA methylation and result in some re-expression of the silenced genes. We have further determined that such genes, in the embryonic setting when in a poised, low transcription, state and marked by PcG constituents alone, can have broad domains of intersecting loops of DNA, in part mediated by the PcG complexes. The addition of DNA methylation in the cancer cell can result in tightening of these loops and a deeper state of non-poised, gene silencing.

Most recently, we have questioned whether the nuclear position of genes may pre-dispose them to and/or accompany, their propensity to acquire abnormal promoter DNA hypermethylation. Studies of others have well documented changes in nuclear organization of chromatin in tumor cells as well as association of aberrant methylation with long range silencing of neighboring genes. Further, certain tumors show a high incidence of promoter methylation termed as the CpG island methylator phenotype (CIMP). We, thus, analyzed the nuclear chromatin architecture and nuclear position of genes in hypermethylated, inactive versus non-methylated, active states and in the absence or presence of long range silencing domains and CIMP. When viewed by combined immunostaining for active/repressive chromatin marks and FISH in
colorectal cancer cell lines we find that aberrant DNA methylation and silencing of such genes appears independent of their being positioned at heterochromatic versus euchromatic nuclear domains. Moreover, whether or not this hypermethylation and silencing is associated or not with long-range epigenetic silencing of neighboring genes is also independent of euchromatic versus heterochromatic locations. Finally, longe range silencing, per se, does not appear to be increased in the CIMP phenotype setting.

We conclude that, in cancer, extensive changes around promoter chromatin of individual genes, or gene clusters, occurs locally, independent of nuclear position and without causing repositioning. We believe these cancer associated findings have important implications for understanding relationships between nuclear organization and gene expression.

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VISITOR INFORMATION

EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (****) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes

Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, west wing, lower level
PIN#: **Press 64365 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -
Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322