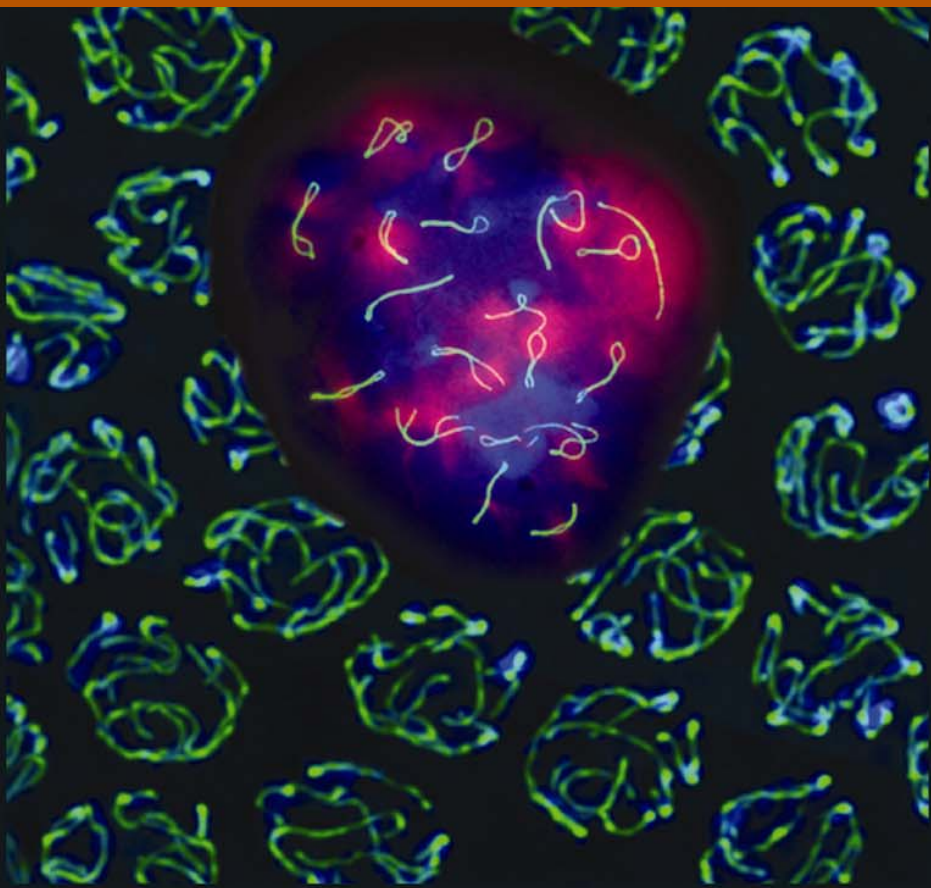
Abstracts of papers presented
at the 2010 meeting on

GERM CELLS

October 5–October 9, 2010



Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

GERM CELLS

October 5–October 9, 2010

Arranged by

John Eppig, *The Jackson Laboratory*
Margaret Fuller, *Stanford University School of Medicine*
Phillip Newmark, *HHMI/University of Illinois at Urbana-Champaign*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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Cover, foreground: Severe asynapsis in a Maelstrom-mutant mouse spermatocyte. Green - SYCP3; Red - phosphorylated gamma-H2AX; Blue - DAPI. Image courtesy of Godfried W. van der Heijden and Alex Bortvin.

Cover, background: A field of *C. elegans* germ cells from the pachytene stage of meiotic prophase, in which homologous chromosome pairs are fully aligned along their lengths. Meiosis-specific chromosome structures known as synaptonemal complexes, which assemble at the interfaces between paired homologs, are depicted in green; DAPI-stained chromatin is depicted in blue. Images are projections of 3D data stacks encompassing whole nuclei. Image courtesy of Michiko Hayashi and Anne Villeneuve.

GERM CELLS

Tuesday, October 5 – Saturday, October 9, 2010

Tuesday	7:30 pm	**1** Germ Cell Specification, Migration and Fate
Wednesday	9:00 am	**2** Germ Line Stem Cells
Wednesday	2:00 pm	**3** Poster Session I
Wednesday	4:30 pm	Wine and Cheese Party *
Wednesday	7:30 pm	**4** Germ Line Stem Cells, Gonocyte Proliferation, and the Switch from Mitosis to Meiosis
Thursday	9:00 am	**5** Transcriptional Control and Epigenetic Programming
Thursday	2:00 pm	**6** Poster Session II
Thursday	7:30 pm	**7** Post-transcriptional Control and Small RNAs in Gametogenesis and Early Embryos
Friday	9:00 am	**8** Meiosis
Friday	2:00 pm	**9** Cell Biology of Germ Cells
Friday	6:00 pm	Concert
	7:00 pm	Banquet
Saturday	9:00 am	**10** Germ Line / Soma Communication and Sex Determination

Poster sessions are located in *Bush Lecture Hall*

* *Airslie Lawn*, weather permitting

Mealtimes at Blackford Hall are as follows:

Breakfast 7:30 am-9:00 am

Lunch 11:30 am-1:30 pm

Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

TUESDAY, October 5—7:30 PM

SESSION 1 GERM CELL SPECIFICATION, MIGRATION AND FATE

Chairperson: **A. Spradling,** Howard Hughes Medical Institute, Carnegie Institution, Baltimore, Maryland

WEDNESDAY, October 6—9:00 AM

SESSION 2 GERM LINE STEM CELLS

Chairperson: **J. Kimble,** Howard Hughes Medical Institute, University of Wisconsin-Madison

WEDNESDAY, October 6—2:00 PM

SESSION 3 POSTER SESSION I

WEDNESDAY, October 6—4:30 PM

Wine and Cheese Party

SESSION 4 GERM LINE STEM CELLS, GONOCYTE PROLIFERATION, AND THE SWITCH FROM MITOSIS TO MEIOSIS

Chairperson: **B. Braun,** The Jackson Laboratory, Bar Harbor, Maine

THURSDAY, October 7—9:00 AM

SESSION 5 TRANSCRIPTIONAL CONTROL AND EPIGENETIC PROGRAMMING

Chairperson: **H. Lin,** Yale University, New Haven, Connecticut

SESSION 6 POSTER SESSION II

SESSION 7 POST-TRANSCRIPTIONAL CONTROL AND SMALL RNAs IN GAMETOGENESIS AND EARLY EMBRYOS

Chairperson: **R. Lehmann,** Howard Hughes Medical Institute, Skirball Institute, NYU School of Medicine, New York

Dazl regulation of RNA processing in mammalian germ cells
Hsu-Hsin Chen, Donald B. Bloch, Cody Tramp, Christa Buecker, Geijsen Niels.
Presenter affiliation: Massachusetts General Hospital, Boston, Massachusetts; Harvard University, Cambridge, Massachusetts. 171

Tdrd1 recruits piRNA targets and Piwi proteins to facilitate piRNA amplification
Hsin-Yi Huang, Saskia Houwing, Lucas Kaaij, Stefan Redl, Amanda Meppelink, Sharon Gauci, Harmjan Vos, Bruce W. Draper, Cecilia B. Moens, Boudewijn Burgering, Peter Ladurner, Jeroen Krijgsveld, Eugene Berezikov, René F. Ketting.
Presenter affiliation: Hubrecht Institute-KNAW & UMC Utrecht, Utrecht, Netherlands. 172

FRIDAY, October 8—9:00 AM

SESSION 8 MEIOSIS

Chairperson: **M.A. Handel,** The Jackson Laboratory, Bar Harbor, Maine

Chromosome dynamics during meiosis in *C. elegans*
David J. Wynne, Ofer Rog, Nicola C. Harper, Sara Jover Gil, Zoe J. Assaf, Abby F. Dernburg.
Presenter affiliation: Howard Hughes Medical Institute, University of California-Berkeley, Berkeley, California. 173

Illuminating formation and regulation of crossovers during *C. elegans* meiosis
Anne Villeneuve, Rayka Yokoo, Simona Rosu, Diana Libuda, Karl Zawadzki.
Presenter affiliation: Stanford University, Stanford, California. 174

Chromatin and meiotic crossover formation
Judith Yanowitz, Cynthia Wagner, Phil Meneely, Olivia McGovern, Frazer Heinis.
Presenter affiliation: Carnegie Institution, Batimore, Maryland; Magee-Womens Research Institute, Pittsburgh, Pennsylvania. 175

FRIDAY, October 8—2:00 PM

SESSION 9 CELL BIOLOGY OF GERM CELLS

Chairperson: **D. Page,** Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge

CONCERT
Grace Auditorium

Diane Walsh, piano

With a reputation as a performer of “romantic sweep and arching lyricism” (The New York Times) and “a pianist with superb technique” (The Boston Globe) Diane Walsh has created an international career of distinction. On disc, as well as in recital, chamber music and concerto performances worldwide, she has brought a clear vision and superlative skill to a broad range of repertoire from Bach to Berg. In addition, her recent work on the theatre stage has further illuminated the ingenuity of her musical gifts.

In September 2007, Diane Walsh joined the cast of 33 Variations, a new play by Moisés Kaufman in its debut production at Arena Stage in Washington, D.C. for a month-long run. The play is focused on the creation of Beethoven's Diabelli Variations, which Ms. Walsh performs on stage throughout the play. The play had a second run at the La Jolla Playhouse in April 2008, and then ran on Broadway from February 9 to May 21, 2009 at the Eugene O'Neill Theater, starring Jane Fonda as the musicologist and with Ms. Walsh as the pianist.

Ms. Walsh has given recitals at such major venues as the 92 Street Y, the Metropolitan Museum of Art, Merkin Concert Hall and the Miller Theatre in New York City, the Kennedy Center in Washington, Orchestra Hall in Chicago, Wigmore Hall in London, the Concertgebouw in Amsterdam, Philharmonic Hall in Leningrad, Dvorak Hall in Prague, and the Mozarteum in Salzburg, among many others. She has appeared in concerto performances with the San Francisco Symphony, the Indianapolis Symphony, the St. Louis Symphony, the Austin, Syracuse and Delaware Symphonies, the American Symphony Orchestra in New York, and the radio symphonies of Munich, Frankfurt, Stuttgart and Berlin. Diane Walsh has toured with Orpheus and the Orchestra of St. Luke’s and given concerto performances with orchestras in Brazil, the Netherlands, the Czech Republic and Russia.

An active recording artist, Ms. Walsh has made fourteen CDs of diverse repertoire for Sony, Nonesuch, Koch, Newport, Stereophile, Composers Recordings, Inc. and Biddulph. For Jonathan Digital Recordings, she has recorded the Beethoven Diabelli Variations, and most recently, volume one of the Complete Schubert Piano Sonatas.

In demand as a chamber musician, she has performed at many festivals including Marlboro, Santa Fe, Bard, Eastern Shore, the International Musician's Seminar in Cornwall, and Strings in the Mountains. She has been guest artist with the Fine Arts, Mendelssohn, Brentano and Lydian String Quartets, and is a member of both the Walsh-Drucker-Cooper Trio, and of La Fenice (comprised of piano, string trio and oboe.) In addition, Ms. Walsh has served as Artistic Director of the Skaneateles Festival in upstate New York. During her long tenure she presented world-renowned performers and designed and performed in innovative programs such as An Evening with Bill Irwin and The Love Letters of Robert and Clara, set to music by the Schumanns.

Among her many honors and awards, Diane Walsh won the Concert Artists Guild International Competition and the Young Concert Artists International Auditions, and was winner of top prizes at both the Munich ARD and Salzburg Mozart Competition, the William Kapell International Competition in Maryland and the Busoni International Competition in Italy. A prizewinner in the Van Cliburn International Piano Competition, she also won that competition's chamber music award.

Diane Walsh is a member of the piano faculty at Mannes College The New School for Music in New York City. She has served on the juries of national and international piano competitions such as the William Kapell, Concert Artists Guild, Young Concert Artists, PianoArts, and the Jovenes Concertistas Brasileiros Competition in Rio de Janeiro. Ms. Walsh is a Steinway Artist.

FRIDAY, October 8

BANQUET

Cocktails 7:00 PM Dinner 7:45 PM

SESSION 10 GERM LINE / SOMA COMMUNICATION AND SEX DETERMINATION

Chairperson: **E. Matunis,** Johns Hopkins University School of Medicine, Baltimore, Maryland

SPECIFICATION OF GERM CELL FATE: HOW IMPORTANT ARE GERM GRANULES?

Geraldine Seydoux, Chris Gallo, Jennifer Wang

Johns Hopkins School of Medicine, HHMI, Department of Molecular Biology and Genetics, Baltimore, MD, 21205

We have analyzed the dynamic behavior of germ granules during *C. elegans* embryogenesis. We find that asymmetric segregation of germ granules to the germline involves both mechanisms that stabilize germ granules in germline blastomeres and mechanisms that disassemble germ granules in somatic cells. We will report on a mutant (*pptr-1*) where germ granule components are segregated equally to both somatic and germline blastomeres. Surprisingly, *pptr-1* mutants retain the ability to specify a germline that uniquely expresses germ granule components during post-embryonic development. Our results suggest that in *C. elegans*, as in mammals, germ granules are a consequence, not a cause, of germ cell fate.

DEVELOPMENT AND REGULATION OF MULTIPOTENT CELLS

Gary Wessel, Eric Gustafson, Mamiko Yajima

Brown University, MCB Department, Providence, RI, 02912

Multipotent cells are capable of differentiating into diverse cell types. In many animals, multipotent cells are initially specified early in development and then are set aside from influence by neighboring cells. Only later in development do they proliferate and differentiate into an array of final cell fates. In several organisms multipotent cells yield both somatic cells and primordial germ cells (Rebscher et al., 2007; Wang et al., 2007; Juliano et al., 2010; Juliano and Wessel, 2010) and may represent an ancient type of stem cell.

Sea urchins are representatives of the deuterostome phyla Echinodermata, an early-branching sister group to the Chordates. These organisms yield millions of embryos with an exposed set of multipotent cells – the small micromeres. These cells divide only once throughout embryogenesis and then proliferate in larval development to form various tissues of the adult rudiment (Cameron et al., 1991; Pearse and Cameron, 1991). The small micromeres selectively accumulate several factors involved generally in germ line fates, such as vasa, piwi and nanos. These factors accumulate by specific transcriptional activity as well as by differential protein turnover. Vasa, for example, is present throughout the early embryo and upon birth of the small micromeres, is degraded in all cells except the small micromeres. An E3-ubiquitin ligase, gustavus, contributes to this selective accumulation through a positive regulatory mechanism similar to what has been seen recently in the regulation of Vasa in the pole cells of Drosophila. Surprisingly, the sea urchin embryo can recover Vasa-positive, multipotent cells in response to removal of the endogenous cells; small-micromere-null embryos develop into normal adults that are gravid (Ransick et al., 1996). Reprogramming of the multipotent cells begins by accumulation of Vasa throughout the embryo - to over 10-fold the level of the endogenous vasa expression by use of the existing maternal mRNA (Voronina et al., 2008) followed by a gradual restriction to a new set of multipotent cells. Thus, all cells of the embryo have the capacity to accumulate Vasa, and some are able to reprogram their fates to include multipotent cells. We hypothesize that the Vasa up-regulation depends on differential regulation of gustavus and possibly other ubiquitylation complexes.

We support the model that the germline molecular program originated in multipotent cells, and was subsequently co-opted by more specialized, primordial germ cells. It will be necessary to collect more functional data from animals spanning diverse taxa in order to reveal the most ancient and essential portions of this proposed multipotency molecular program.

GERM CELL SPECIFICATION IN MICE IN VIVO AND IN VITRO

Mitinori Saitou[1,2]

[1]Kyoto University Graduate School of Medicine, Department of Anatomy and Cell Biology, Kyoto, 606-8501, Japan, [2]JST, CREST, Kyoto, 606-8501, Japan

The specification of germ cell fate in development initiates mechanisms essential for the perpetuation of genetic and epigenetic information across the generations. In mice, germ cell fate is induced in a subset of pluripotent epiblast cells during early gastrulation. Our studies have shown that germ cell specification requires at least three key molecular/cellular events: repression of the somatic program, re-acquisition of potential pluripotency, and an ensuing genome-wide epigenetic reprogramming. Blimp1 and Prdm14 are the two critical transcriptional regulators that orchestrate these events. We have also revealed a signaling principle that confers the germ cell fate to the epiblast cells, underscoring the feasibility of generating the mammalian germ cell lineage in vitro. I would here like to present our recent findings regarding the induction of germ cell fate from pluripotent stem cells in culture.

TRANSCRIPTIONAL REGULATION DURING GERM LINE STEM CELL DIFFERENTIATION

Ruth Lehmann[1], Prashanth Rangan[1], Colin Malone[1,4], Caryn Navarro[1,2], Vitor Barbosa[1,3], Greg Hannon[4]

[1]NYU School of Medicine, HHMI and Skirball Institute, New York, NY, 10016, [2]Boston University School of Medicine, Department of Medicine/Genetics Program, Boston, MA, 02118, [3]Instituto Gulbenkian de Ciência, Genetics, Oeiras, 2780-156, Portugal, Cold Spring Harbor Laboratory, Watson School of Biological Sciences and HHMI, Cold Spring Harbor, NY, 11724

In most species studies, primordial germ cells (PGCs) require transcriptional silencing for their proper specification. In many stem cell systems it has been shown that transcriptional silencing mechanisms maintain stemness and represses differentiation. Here we show that transcriptional silencing during Drosophila oogenesis is required in the germ line stem cell (GSC) progeny in order to exit the stemcell program. Mutants defective in transcriptional silencing, such as histone demethylase Su(var)3-3/dLsd1, histone methyltransferase (egg/dSETDB1), and polar granule component (pgc) are differentiation defective. This transcriptional silencing in the progeny of GSCs is evidenced by an increase of repressive marks and the movement of Su(var)3-3/dLsd1 and egg/dSETDB1 from the cytoplasm of GSC to the nucleus of the progeny. We identify transposable elements and the major piRNA producing loci as the primary targets of this transcriptional repression. Our data suggest that repressive marks on the piRNA producing clusters control of transposons expression and the transition from germ line stem cell to differentiating gamete.

GERM CELL MOTILITY AND DIRECTED MIGRATION IN ZEBRAFISH

Erez Raz

University of Muenster, Institute for Cell Biology, Muenster, 48149, Germany

The migration of zebrafish primordial germ cells (PGCs) is directed by SDF-1a and serves as a model for long-range chemokine-guided cell migration in development. PGCs acquire competence to respond to the chemokine following discrete maturation steps that depend on the function of the RNA-binding protein Dead end. The mechanisms by which Dead end facilitates cell motility and migration competence will be presented. Following the acquisition of cell motility, the germ cells migrate directionally towards their target - the somatic part of the gonad. This migration is guided by the chemokine SDF-1a, while cues encoded by the highly related chemokine molecule SDF-1b are practically ignored. The mechanisms responsible for the ability of the PGCs to discriminate between the two signals will be described, as well as the mechanisms controlling the formation of an informative SDF-1a gradient.

THE ECONOMICS OF REPRODUCTION

Marc Van Gilst

Fred Hutchinson Cancer Research Center, Seattle, WA, 98109

Within each of us, a complex metabolic networks help optimize nutritional input to meet the demands of growth, reproduction, and cognition. In essence, this fundamental characteristic of most metazoans is a matter of basic economics, and a major focus of our laboratory is to characterize and quantify economic systems important for biological function and flexibility. An ideal system for investigating this model is the *C. elegans* reproductive mechanism. Under favorable conditions, a single *C. elegans* hermaphrodite can produce more than 250 offspring in five days. However nutrient shortages can stimulate worms to slow or even arrest reproduction via a variety of mechanisms. Investigating reproductive potency and flexibility in worms led us to identify the nuclear receptor NHR-49 as a key factor for tuning reproductive rate to match nutrient supply. Using the economic analogy, NHR-49 acts as a chief economist, globally balancing supply and demand by working at the local level with a team of NR "specialists" to alter the infrastructure of the supply side chain while controlling demand by regulating the rate of ovulation and oocyte maturation. Our recent investigations have revealed numerous links in the supply side chain regulated by NHR-49, many of which have not yet been described for nuclear receptors. Our work not only supports the usage of economic principles for interrogating biological processes, but has also revealed numerous links in the supply side chain that can be causal factors in aging and disease when their capacity is disrupted or overloaded. The principles we have characterized thus far are widely applicable to a variety of biological systems.

REGULATION OF CENTROSOME ORIENTATION IN ASYMMETRIC DIVISION OF THE DROSOPHILA MALE GERM LINE STEM CELLS.

Yukiko M Yamashita, Hebao Yuan, Mayu Inaba, Therese M Roth, Ason Chiang, Viktoria Salzmann

University of Michigan, Life Sceinces Institute, Center for Stem Cell Biology, Ann Arbor, MI, 48109-2216

Adult stem cells are the source of continuous supply of differentiated cells. Upon stem cell division, its daughters either self-renewal or differentiate, a balance of which is critical to tissue homeostasis. To maintain this critical balance, many stem cells have a potential to divide asymmetrically, giving rise to one stem cell and one differentiating cell.

Drosophila male germ line stem cells (GSCs) divide always asymmetrically, producing one self-renewed stem cell and one differentiating cell. This asymmetric stem cell division is tightly controlled via stereotypical positioning of mother and daughter centrosomes, leading to oriented spindle with respect to the stem cell niche and thus asymmetric stem cell division.

We now show that GSCs possess a novel checkpoint that monitors correct centrosome orientation prior to mitosis, ensuring asymmetric outcome of the division, and identify Par-1 kinase, DE-Cadherin and centrosomin as being involved in this checkpoint. We further show evidence that this checkpoint is the basis for cell cycle slow down in response to limited availability of nutrients: we have found that GSC division slows down by misorienting centrosomes, which in turn activates the centrosome orientation checkpoint, in response to the limited nutrients availability. In the mutant of the centrosome orientation checkpoint, GSCs failed to slow down the cell cycle even under the limited nutrients condition. Together we propose that GSC has evolved elaborate mechanisms to precisely regulate the asymmetry and frequency of the division.

THE CCR4 DEADENYLASE IS REQUIRED WITH NANOS AND PUMILIO FOR GERMLINE STEM CELL SELF-RENEWAL IN *DROSOPHILA*

Martine Simonelig, Willy Joly, Isabelle Busseau

Institute of Human Genetics-CNRS, Genetics and Development, Montpellier, 34396 cedex 5, France

In the *Drosophila* female germline, two or three germline stem cells are localised at the anterior-most region -the germarium- of each ovariole. Each stem cell divides asymmetrically to self-renew and to produce a cell that differentiates in a cystoblast.
Nanos (Nos) and Pumilio (Pum) are two intrinsic stem cell factors that are required for the maintenance of germline stem cells, by preventing their differentiation. Therefore, stem cell maintenance corresponds in part to the repression of the differentiation program. Nos and Pum are RNA binding proteins and translational repressors, indicating that translational control has an important role in the switch between germline stem cell self-renewal and differentiation. However, the mechanism of action of Nos and Pum in these cells is unknown. In the embryo, Nos and Pum can act using two different mechanisms: i) inhibition of translation initiation and ii) activation of deadenylation, in which Nos and Pum directly recruit the CCR4 deadenylation complex onto a specific mRNA.
To investigate the molecular mechanisms of translational repression by Nos and Pum in the germline stem cells, we analysed the function of the CCR4 deadenylase in these cells. CCR4 is encoded by the *twin* gene. We observe the loss of germline stem cells in *twin* mutant ovaries, consistent with the role of CCR4 in the maintenance of stem cells. Clonal analysis of *twin* mutant germline stem cells shows that *twin* function in the stem cells is cell-autonomous. Cell-specific (somatic/germline) rescue experiments of *twin* mutants with the *twin* transgene are also consistent with this. We find that *twin* interacts genetically with *nos* and *pum*, as the removal of one copy of *nos* or *pum* in *twin* hypomorphic mutants leads to an accelerated loss of germline stem cells. Moreover, co-immunoprecipitation experiments reveal that Nos, Pum and CCR4 proteins physically interact in germline stem cells. These results strongly suggest that CCR4, Pum and Nos act together in the maintenance of germline stem cells. We propose that the CCR4/Nos/Pum complex represses translation of differentiation factor mRNAs in the germline stem cells by promoting their deadenylation. This would occur through the direct recruitment of the CCR4 deadenylase by Nos and Pum onto these specific mRNAs. Identification of differentiation factor mRNAs potentially targeted by this complex is in progress.
Our results reveal the essential role of CCR4-mediated deadenylation in the self-renewal of germline stem cells and identify a molecular mechanism of action of Nos and Pum in this process.

REGENERATION OF THE GERMLINE BY SELF-RENEWING GSCS IN THE BASAL CHORDATE, *BOTRYLLUS SCHLOSSERI*

Anthony De Tomaso

UC Santa Barbara, MCD Biology, Santa Barbara, CA, 93106

We are studying germline regeneration in the colonial ascidian, *Botryllus schlosseri*. Ascidians are basal chordates and represent the transition phylum between invertebrates and vertebrates. Ascidian embryogenesis results in a motile chordate tadpole larvae which later metamorphoses into a sessile invertebrate adult. In addition, *B. schlosseri* belongs to a subset of ascidians which are colonial and grow, not by increasing in size, but via a budding process which results in a colony of genetically identical individuals (called zooids) united by a common vasculature. Each zooid is an independent body, with its own somatic and germline tissues, and each week gives rise to 1-4 new buds which originate in a group of stem cells which can be prospectively enriched and transplanted. We are focused on understanding the source of germline regeneration during this budding process. Recent results suggest that long-lived germline stem cells (GSCs) are specified during embryogenesis and contribute to germline formation each week for the life of a genotype (6 mo- >2 yrs). Following metamorphosis, colonies undergo 6-12 budding cycles prior to the appearance of gonads, and in addition adults often cycle between fertile and infertile states. GSCs isolated from juvenile or non-fertile colonies can contribute to germline formation immediately following transplant to a sexually mature recipient. In addition, it has been found that allogeneic transplantation of GSCs can lead to competition between genetically distinct stem cell lineages within a chimera, often leading to complete replacement of the germline over time by a single genotype. This process, called germ cell parasitism, is a repeatable and heritable trait, and winner and loser genotypes can be found in lab-reared strains and natural populations. Parasitic abilities are autonomous to the GSCs themselves, and retained upon experimental transplantation. We have recently found that > 90% of the testes isolated from individual zooids within a chimeric colony are clonal, and analyzed the kinetics of chimerism over time. We have also found that fertility can be induced in a juvenile colony following parabiosis to a fertile adult, and that the outcome depends on the size ratio of the two individuals. Together, these results suggest GSCs are present in an infertile colony, that fertility in a colony is likely due to niche formation and/or GSC activation due to a global signal, and that the basis of stem cell parasitism is homing. We are currently identifying differentially expressed genes between fertile and infertile colonies as well as characterizing GSCs prospectively enriched from winner and loser parasitic genotypes using mRNA-seq. Current results will be presented.

SPERMATOGONIAL STEM CELLS REGULATE THEIR NICHE

Robert E Braun, Manju Sharma

The Jackson Laboratory, Mammalian Genetics, Bar Harbor, ME, 04609

Murine spermatogenesis is maintained by a population of spermatogonial stem cells (SSC) that lie on the basal lamina (basement membrane) of the seminiferous tubules. Marker analysis, lineage tracing and in vivo imaging support the hypothesis that SSCs are a heterogeneous population of cells with different degrees of stem cell potential. During steady-state spermatogenesis, the most undifferentiated type A single spermatogonia (As) support the bulk of spermatogenesis. However, during regeneration of the stem cell compartment following depletion of SSCs (e.g. irradiation or chemical stress), or during repopulation following SSC transplantation to a germ cell-less testis, pairs of type A spermatogonia (Apr) and chains of type A aligned spermatogonia (Aal) are able to contribute to renewal of the SSC compartment. This plasticity underlies the robustness of the stem cell compartment. SSCs are in direct contact with the extracellular matrix (ECM) of the basal lamina and with the Sertoli cells of the seminiferous epithelium. Glial Cell Line-Derived Neurotrophic Factor (GDNF) is expressed by Sertoli cells and is essential for maintenance of SSCs, which express the GDNF Family Receptor α 1 (GFRα1) on their cell surface. Expression of GDNF is periodic during the cycle of the seminiferous epithelium and is elevated upon depletion of the SSC population. Forced expression of GDNF throughout the cycle induces formation of large nests of functional SSCs. Cell cycle analysis during steady state spermatogenesis and during regeneration indicates that GDNF promotes self-renewal of SSCs by blocking their differentiation. Nest formation is accompanied by reorganization of the ECM, suggesting that SSCs are capable of inducing the formation of ECM. We propose the SSCs are active players in creation of the niche and that the ECM, perhaps as acting as a reservoir for GDNF and other molecules, regulates stem cell self-renewal.

PLZF REGULATES GERMLINE PROGENITOR SELF-RENEWAL BY OPPOSING MTORC1

Robin M Hobbs[1], Marco Seandel[2], Ilaria Falciatori[3], Shahin Rafii[3], Pier Paolo Pandolfi[1]

[1]Cancer Genetics Program, Beth Israel Deaconess Cancer Center, Departments of Medicine and Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02115, [2]Department of Surgery, Weill Cornell Medical College, New York, NY, 10065, [3]Howard Hughes Medical Institute, Department of Genetic Medicine, Weill Cornell Medical College, New York, NY, 10065

Maintenance of male germline progenitors (spermatogonial progenitor cells; SPCs) in the mouse requires expression of the transcription factor Plzf although the relevant mechanisms are poorly understood. Here we show that SPCs lacking expression of Plzf have enhanced activity of the mTORC1 signaling complex, a critical regulator of cell growth and stem cell function. Aberrant mTORC1 activation in *Plzf*$^{-/-}$ SPCs inhibits their response to GDNF, a key niche-derived growth factor required for self-renewal, via a negative feedback effect at the level of the GDNF receptor. We find that Plzf opposes activity of the mTORC1-signaling complex in SPCs by enhancing expression of the mTORC1 inhibitor Redd1. Importantly, we observe that inhibition of mTORC1 activity with Rapamycin treatment *in vivo* attenuates the SPC maintenance defect of *Plzf* $^{-/-}$ mice, demonstrating that the mTORC1 pathway is a key target for Plzf in maintaining SPC function. We therefore define an important mechanism linking Plzf expression and mTORC1 activity with the ability of SPCs to respond to self-renewal stimuli and propose a model whereby negative feedback from mTORC1 to the GDNF receptor balances SPC growth with self-renewal.

GERM PLASM FORMATION IN ZEBRAFISH

Franck BONTEMS[1], Roland DOSCH[1,2]

[1]Université de Genève, Dept. de Zoologie et Biologie Animale, Geneva, 1211, Switzerland, [2]Georg-August Universität Göttingen, Abt. Entwicklungsbiochemie, Göttingen, 37077, Germany

Germ plasm acts as a cytoplasmic determinant, which controls the differentiation of germ cells during animal development. Whereas in vertebrates some aspects of germ plasm composition and its biochemical function such as piRNA generation have been characterized, the regulators of germ plasm assembly are unknown.

Analysis of the *bucky ball* (*buc*) mutant in zebrafish shows that the *buc* gene is required for germ plasm assembly in oocytes. We positionally cloned *buc*, which encodes a novel vertebrate specific protein. In addition, the expression of Buc is consistent with a role in germ plasm organization. Furthermore, the protein localizes to the cleavage furrows of the 4-cell embryo, which originates the primordial germ cells in zebrafish. Interestingly, overexpression of Buc induces the formation of additional germ cells by recruiting germ plasm components. We conclude that Buc is the first germ plasm factor, which is necessary and sufficient for germ plasm formation in vertebrates.

To further elucidate the molecular mechanisms of germ plasm assembly, we have generated a Buc-GFP transgenic line. This line generates functional Buc, since it rescues the mutant phenotype. Moreover, it allows now to continuously observe germ plasm dynamics from early oogenesis to early embryogenesis. Moreover this line will be a good tool to study the biochemical function and cell biological role of Buc and thus, provides an entry point to molecularly dissect germ plasm formation in vertebrates.

GERMINAL CRADLES EXPRESSING *SOX9B* HARBOR GERMLINE STEM CELLS IN THE OVARY OF TELEOST FISH, MEDAKA

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Neo-oogenesis in the vertebrate ovary has been a topic of long standing broad interest. We show the direct evidence that germline stem cells are present in the ovary of teleost fish, medaka (*O.latipes*). The germline stem cells express *nanos2* and are colonized in the region, named germinal cradles, which are within the cellular networks of *sox9b*-expressing cells. The networks are present in the germinal epithelium in medaka ovary, histologically equivalent to the surface epithelium in mammalian ovary. By clonal analysis with Cre/loxP system, we revealed that *nanos2*-expressing cells produce cystic germ cells, dividing three to five times, to result in the meiotic germ cells. Some germ cells are lost in the cysts but the remaining ones reach diplotene stage. The diplotene oocytes are indivisualized to move to the stroma compartment, which then become all the subsequent stages of oocytes and fertile eggs. We confirmed that they are finally transmitted to the next generation. This process is reminiscent of germarium in the ovary of *Drosophila*. Oocytes depletion experiments suggest at least two regulatory steps present in the ovary to generate mature eggs.
Interestingly mammalian homologue, *sox9*, is only detected in the testis and essential for testicular development. We have analyzed the function of *sox9b* gene using mutant medaka and will mention the novel function of *sox9b* on the germ cells.

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DIFFERENTIATION PROPENSITY OF HUMAN EMBRYONIC STEM CELL AND INDUCED PLURIPOTENT STEM CELL LINES TO THE GERM CELL LINEAGE

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Infertility is a common health problem affecting 10 to 15 percent of couples according to the Center for Disease Control and Prevention, and poor germ cell quality is one of the major causes. Understanding normal germ cell development will help us improve current infertility treatments, but direct analysis of human germ cell development has been difficult due to inaccessibility of germ cells during fetal development. Both human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have been shown to differentiate into primordial germ cells (PGCs), providing an invaluable model to study germ cell development, although it is unknown whether these stem cells could differentiate into mature and functional germ cells *in vitro*. It has also been shown that there are marked differences in differentiation propensity among distinct stem cell lines. However, direct comparison of the differentiation potential of human stem cell lines to the germ cell lineage has not been reported to date. Here, we compare the ability of 11 human ESC and iPSC lines to differentiate to the germ cell lineage using spontaneous differentiation techniques. The goal is to better understand human germ cell development, specifically female germ cell differentiation, by determining the capacity of distinct human pluripotent stem cell lines to differentiate to the germ cell lineage. We analyze the expression profile and protein expression of VASA and NANOS3 (germ cell-specific markers). We hypothesized that distinct human pluripotent stem cell lines, with various genetic backgrounds and derivations, differ in their ability to differentiate towards the germ cell lineage. Our preliminary data support our hypothesis, as different human pluripotent stem cell lines express distinct levels of germ cell markers. Characterization of differentiation potential of distinct cell lines will help elucidate the effects of genetic backgrounds on germ cell differentiation and development.

CHROMATIN DYNAMICS DURING THE ESTABLISHMENT OF THE PIG GERM LINE

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Epigenetic reprogramming is critical for genome regulation during the development of the germ line. Genome wide demethylation in mouse primordial germ cells (PGC) is a unique reprogramming event critical for erasing epigenetic memory and preventing the transmission epimutations to the next generation. Erasure of gender specific imprints and partial demethylation of repetitive elements occurs in mouse germ cells colonizing the gonad. In addition to DNA demethylation, a major reprogramming of histone marks occurs, and many of these changes are concurrent with a G2 arrest. There is limited information on how well conserved these events are in other mammals, therefore here we report on the dynamic reprogramming of DNA methylation at imprinted loci, DNA repeats and global changes in histone H3K27me3 and H3K9me2 in the developing germ line of the domestic pig. Analysis of IGF2-H19 regulatory region showed gradual demethylation in PGC soon after reaching the gonad (E22) and continued until E42. The DMR2 of IGF2R locus, in contrast, showed precocious demethylation in males migratory PGC before E22. Furthermore, in female embryos IGF2R demethylation was delayed until E29-31, suggesting gender specific differences during the erasure of the IGF2R. Analysis of DNA repeats (SINE and LINE1) showed gradual demethylation from E25 to E29-31, and these repeats became de novo methylated by E42. Finally we investigated changes in histone marks in migrating PGC and established that strong H3K27me3 staining was present in migratory PGC between E15 and E21. In contrast, H3K9me2 signal was low in PGC by E15 and completely erased by E21. Cell cycle analysis of gonadal PGC (E22-31) showed a typical pattern of cycling cells, however, migrating PGC (E17) showed an increased in the proportion of cells in G2.
Our study demonstrates that epigenetic reprogramming of histone H3K9me2 and H3K27me3 detected between E15-E21 precedes the dynamic DNA demethylation at imprinted loci and DNA repeats between E22-E42. These findings demonstrate that major epigenetic reprogramming of the pig germ line follows the overall dynamics shown in mice, suggesting that epigenetic reprogramming of PGC is conserved in mammals. Although the time required for accomplishing these events is more than three times that required in mice, the dynamic reprogramming occurs at equivalent developmental stages to rodents mice, indicating that the difference probably stems from the fact that development is slower in the pig. Interestingly, the extended window of time has highlighted the asynchrony in the demethylation of certain genomic loci on a gender specific manner.

INVESTIGATING THE ROLE OF WEE-1.3 IN *C. ELEGANS* OOCYTE MATURATION.

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Meiosis is a specialized cell cycle essential in all species for the generation of functional gametes. The female gamete, or oocyte, becomes arrested early in development typically during meiosis I, and must be reactivated at a later time point in order to ensure fertilization. This reactivation is termed oocyte meiotic maturation and is required in all female species. We are using the genetic model system of the nematode, *C. elegans*, to investigate the mechanisms behind oocyte meiotic maturation. Our lab has previously shown that an inhibitory kinase of the cell cycle, WEE-1.3, that has mammalian orthologs, is required for proper timing of oocyte meiotic maturation. Upon depletion of WEE-1.3 via RNA interference oocytes mature precociously and though the oocytes encounter sperm, they are not fertilized and the worm becomes sterile. The mechanism by which *wee-1.3* RNAi results in precocious oocyte maturation, potential genetic interactors with *wee-1.3* and the subcellular localization of wild type WEE-1.3 remain unknown.

To begin to address these questions we generated multiple transgenic worm lines expressing WEE-1.3 fused to a fluorescent tag to determine the protein's in vivo expression pattern and will report our localization findings. In order to determine genes that interact with *wee-1.3*, we performed an RNAi suppressor screen utilizing 1874 embryonic lethal clones from the OpenBiosystems RNAi library. From this screen we identified 149 genes that when depleted in conjunction with depletion of WEE-1.3 result in a restoration of fertility. We have begun to confirm the identified suppressors and to characterize the mechanisms through which they suppress the sterility of WEE-1.3 RNAi. The strength of this approach is its potentiality for identifying novel components of both the cell cycle and oocyte meiotic maturation

RETINOIC ACID AND ITS TARGET GENE STRA8 COUPLE SPERMATOGONIAL DIFFERENTIATION WITH MEIOTIC ENTRY.

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Retinoic acid (RA) is known to govern two germ cell transitions in mammalian spermatogenesis:
1) the transition from undifferentiated spermatogonia to differentiating spermatogonia, which continue to proliferate mitotically, and
2) the transition from mitosis to meiosis.
These conclusions arise from studies of male rodents maintained on a Vitamin A Deficient (VAD) diet. In VAD rat testes, germ cells arrest at two stages: as undifferentiated spermatogonia, and at the brink of meiotic initiation (1). In VAD mouse testes, germ cells arrest exclusively as undifferentiated spermatogonia (2). In both VAD rats and mice, injections of RA or retinol (the immediate precursor of RA) restore normal spermatogenesis (3, 4).
The architecture of germ cell development in the rodent testis is such that the two RA-governed transitions occur in immediate physical proximity, suggesting the possibility that a single pulse of RA could regulate both steps. The Stra8 (Stimulated by Retinoic Acid 8) gene is induced by RA and is required for the transition from mitosis to meiosis in both sexes in mice (5-7). Our ongoing studies of Stra8-deficient testes indicate an additional role for Stra8, at the transition from undifferentiated spermatogonia to differentiating spermatogonia. These findings lead us to suggest that Stra8 is an important target gene and mediator of the RA signal at both RA-governed transitions in spermatogenesis.

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ADULT WORMS PROTECT GERMLINE STEM CELLS AND EXTEND THEIR REPRODUCTIVE SPAN IN RESPONSE TO STARVATION

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In response to various suboptimal environments, many species of animals can form a diapause, a reversible state of dormancy that facilitates survival during unfavorable times. We observe that adult C.elegans can establish a diapause in response to starvation, which we have termed 'Adult Reproductive Diapause' (ARD). During ARD, the adult germline responds in a unique way: a small population of approximately 35 germ cells is preserved while the majority of the germline is killed off. As a result of this adaptation, starved-then-fed adult worms can regenerate a fully functional germline and produce healthy offspring much later in life, long after an always-fed worm would even be alive.
Both the adult germline and soma exhibit extreme plasticity during and upon recovery from ARD. We have therefore begun characterizing the changes occurring within the stem cell niche and recipient germ cells that enables adult germline stem cells (GSCs) to survive such prolonged starvation. To that end, we are determining the cell cycle properties of the surviving population of germ cells and visualizing the remodeling of the somatic tissues that comprise the stem cell niche in adult worms. Additionally, transmission electron microscopy has revealed many tissue- and cell-specific adaptations occurring in the arrested adult reproductive tract, implicating unique starvation responses that may facilitate GSC survival during ARD. Understanding how adult GSCs and the surrounding niche adapt to starvation will provide insight into the mechanisms employed by stem cell populations to withstand nutritional stress. In this way, similar strategies employed by stem cells can be exploited to attack unwanted stem cell populations (i.e. cancer stem cells) or to protect valuable stem cell populations (i.e. female germline stem cells).

IDENTIFICATION AND ANALYSIS OF TUDOR PROTEIN-ASSOCIATED COMPONENTS OF GERM GRANULES IN DROSOPHILA OVARY

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Germ cells in many animals contain ribonucleoprotein particles, germ granules [1]. Composition of these evolutionarily conserved granules and their functional role are not well understood. In various organisms Tudor domain-containing proteins are the crucial germ granule components. In Drosophila, Tudor protein contains 11 Tudor domains – protein-protein interaction modules which bind to methylated amino acids of target proteins, for example, Piwi family proteins. Here, we used a biochemical approach to identify Tudor-associated proteins in Drosophila ovary. Tudor protein complex was stabilized by chemical crosslinking, purified, and analyzed by mass spectrometry. In addition to Piwi protein Aubergine, we identified DEAD-box RNA helicase and cytoskeleton proteins as specifically present in Tudor complex. Structure-function analysis of Tudor-associated proteins is in progress and will be discussed. Our data suggest that multiple Tudor domains of Tudor protein are redundantly used for interaction with the same protein and also specifically required for trafficking and localization of germline determinants.

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A TRANSIENT NICHE IN THE *DROSOPHILA* OVARY MAINTAINS GERMLINE STEM CELL PRECURSORS IN AN UNDIFFERENTIATED STATE

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Stem cells are characterized by the ability to self-renew and produce numerous differentiated daughter cells. These properties are acquired when stem cell precursors enter a special microenvironment called “niche”. Prior to niche formation, stem cell precursors must remain undifferentiated; however how such precursors maintain their undifferentiated state is largely unknown.
In the *Drosophila* ovary, undifferentiated precursors of germline stem cells (GSCs) are called primordial germ cells (PGCs). A portion of PGCs starts differentiation in the late larval ovary, before niche formation. Most of the PGCs located in the anterior half of the ovary, however, remain undifferentiated during this period, then acquire contact with the nascent niche, and eventually become GSCs. We have identified a novel membrane protein Patch paste (PAP), which is required for GSC formation. PAP is transiently expressed in the late larval ovary on the cell membrane of somatic cells contacting PGCs, with a higher expression in the anterior region of the larval ovary. In *pap* mutant, many PGCs that normally contact somatic cells with high PAP expression start differentiation prior to niche formation. This indicates that PAP is required non-cell autonomously to maintain PGCs in an undifferentiated state. In *pap* mutant the number of GSCs that form after niche formation is reduced. We propose that a transient niche made by the somatic cells maintain GSC precursors in an undifferentiated state, and that PAP is an essential functional component of this niche to ensure their potential to become GSCs.

MEIOTIC DRIVE BY THE MOUSE *T*-HAPLOTYPE

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In general, the germ cells of an individual have equal fertilization potential, resulting in the inheritance of homologous chromosomes at Mendelian ratio. One of the most prominent exceptions to this rule is associated with the *t*-haplotpe of the mouse: Male mice heterozygous for this variant of chromosome 17 produce mutant (*t*-) sperm that by far (typically close to 100%) outcompete wild-type sperm in fertilization, resulting in a prevalence of *t*-bearing offspring. Several *t-complex-Distorter* genes (*Tcd´s*) additively enhance *t*-transmission by interacting with a single *t-complex Responder* gene (*Tcr*).
Our recent analysis shows that the *Tcr* gene product is retained in the cells of origin. Thus, Tcr is able to cause phenotypic differences between *t* and + sperm from *t*/+ males by escaping the general mechanism of gene product sharing between developing sperm cells.
We also have isolated several of the *Tcd* genes, and will present results of their ongoing characterization. *Tcd´*s appear to be expressed from early stages of sperm development onward and their products shared between meiotic partners. *Tcd´*s identified so far encode modifiers of Rho small G-proteins.
We propose the model that theTcd´s deregulate Rho signalling leading to hyper-activation of the wild type form of Tcr, Smok1 (Sperm motility kinase 1) and axonemal dysfunction. Since *Tcd* products are shared between meiotic partners both, *t*- and wild-type cells are compromised. *Tcr* encodes a dominant-negative variant of Smok1, able to counterbalance Smok1 hyper-activation to a level permitting normal sperm function. Since Tcr is restricted to sperm cells carrying the gene, only *t*- sperm are rescued and preferentially fertilize the oocytes.

NANOS1 MAINTAINS GERMLINE STEM CELLS IN THE ADULT ZEBRAFISH OVARY.

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Germline development is a stem cell-driven process in many invertebrate and vertebrate model organisms. In the fly, nanos is required to maintain female germline stem cells (GSCs) by regulating entry into the differentiation program. In addition, homologs of nanos have been shown to be important for the maintenance of GSCs in both the worm and the mouse testis. Our lab has previously reported that nanos1 (nos1) is required to maintain oocyte production in the adult zebrafish. Adult zebrafish ovaries contain germ cells at all stages of development and are capable of producing hundreds of eggs on a weekly basis for their entire adult lifespan. By contrast, nos1 mutant females, while initially fertile, become sterile as young adults. To determine when nos1 is required for oocyte production, we have analyzed mutant ovaries at earlier time points. Oocyte prodution in wild-type ovaries begins around 18 days post fertilization (dpf), and by 35 dpf the earliest stage germ cells, which are 7-10 μm in diameter, are restricted to a lateral domain we have termed the germinal zone. The germinal zone contains both mitotic germ cells and germ cells that are actively entering meiosis. In contrast to wild-type, 35 dpf nos1 mutant ovaries do not contain 7-10 μm germ cells. To determine in what cells nos1 function is required, we have produced germline mosaics. Our results show that nos1 is required cell-autonomously to maintain the 7-10 μm germ cells. In addition, wild-type cells are able to repopulate the germinal zone in a nos1 mutant background. These observations support the hypothesis that the germinal zone is a stem cell niche. We have characterized the transcriptome of the 7-10 μm germ cell population using mRNA-Seq. We produced mRNA-Seq libraries (3 biological replicates) from wild-type and nos1 mutant whole ovary tissue, respectively. Transcripts found in the wild-type, but not the mutant, libraries should be specific to the 7-10 μm cell population. We identified approximately 8000 genes that are expressed in the wild-type ovary. Of these, 200 genes were significantly upregulated in wild-type versus nos1 mutant tissue, and we chose 29 of these genes for further analysis based on their function in processes important for stem cell maintenance (i.e. cell signaling, transcription, extracellular matrix, etc.). In situ hybridization reveals that some of these transcripts are specific for the 7-10 μm cell population.

ROUND SPERMATIDS LACKING THE FIRST BROMODOMAIN OF BRDT EXHIBIT ABERRANT CHROMOCENTER FORMATION AND DIFFERENTIATION INTO SPERM THAT IS GENETIC BACKGROUND-DEPENDENT.

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Brdt, a testis-specific member of the BET family of double-bromodomain containing proteins, is required for spermiogenesis. Mice homozygous for a mutation lacking the first bromodomain are sterile and have few and morphological abnormal sperm. The severity of the phenotype varied somewhat from mouse to mouse. As the original mutant mice were maintained on a mixed genetic background of C57BL/6J (B6) and 129/SvEv (129), we asked whether genetic background could affect the phenotype. We therefore backcrossed the mutation onto pure B6 and 129 backgrounds and found a striking difference in the mutant phenotype: although homozygous mutant mice of both backgrounds are sterile, the phenotype is more severe on the 129 background. In the 129 mutant testes, all tubules and all elongating spermatids in those tubules are morphologically abnormal. In contrast, ~50% of tubules in the B6 mutant testes are grossly morphologically normal and all tubules contain some spermatids which appear to be undergoing, or have undergone, normal elongation. Flow cytometry of propidium iodide stained cells showed that fully condensed sperm are always detected in B6 mutant testes, although on average at 4-fold less than in wild-type. Sperm are difficult to see in the 129 mutant testicular tubules, the epididymides are nearly empty, and sperm counts on both caudal epididymides showed an average of $4.02x10^5$ total sperm, which was 20-fold below the bottom threshold for fertility ($8x10^6$). We also observed an interesting feature of the $Brdt^{\Delta BD1}$ phenotype that involves fragmentation of the chromocenter, a dense region of centromeric heterochromatin unique to round spermatids. This fragmentation was also more severe and penetrant in 129 mutant testes as compared to B6. H1fnt, a spermatid-specific H1 variant required for elongation and condensation, is normally localized at the apical pole of the spermatid nucleus, but is ectopically distributed in mutant strains of mice that exhibit fragmented chromocenters. We observed this ectopic localization of H1fnt in $Brdt^{\Delta BD1}$ mutant spermatids with fragmented chromocenters, which may explain in part the loss of heterochromatin foci previously observed in mutant spermatids. We hypothesize that disruption of proper chromatin architecture in the $Brdt^{\Delta BD1}$ testes leads to aberrant condensation and elongation of spermatids which renders them sterile. Whether this is due to a direct function of Brdt in creating proper chromatin architecture or indirectly, with Brdt functioning in the regulation of genes involved in chromatin organization is being addressed by microarray analysis. [This work was supported by an NIH grant to DJW, GM081767].

EVOLUTIONARILY CONSERVED VSELS IN OVARIAN SURFACE EPITHELIUM SPONTANEOUSLY DIFFERENTIATE INTO OOCYTE-LIKE STRUCTURES, ES-LIKE COLONIES AND EMBRYOS *IN VITRO*

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The central dogma that a female is born with fixed number of oocytes has recently been challenged by presence of very small embryonic-like stem cells (VSELs) in mammalian ovaries. However, discordance exists in the biology of germ cell renewal and mechanisms resulting in post-natal oogenesis with some hypothesizing that human ovarian surface epithelium is a source of bipotent progenitors for both germ cells and granulosa cells and others proposing the presence of pluripotent stem cells in human and mice ovarian surface epithelium.

Present study was aimed at exploring presence of stem cells with embryonic characteristics in adult mammalian ovarian surface epithelium (OSE) of rabbit, sheep, monkey and humans. Stem cells 2- 5µm in size were prominently observed in OSE scrapings in all species and on culture for 3 weeks underwent spontaneous differentiation into oocyte-like structures, embryonic-stem cell like colonies, embryoid bodies, embryo-like structures and cells with neuronal phenotype. Pluripotent transcripts were detected in scraped OSE cells in sheep (Oct-4 & STAT-3) and human (Oct-4, Sox-2, Nanog & TERT) tissue, whereas germ cell transcripts (c-KIT & Oct-4) were detected in 3 week human cultures by RT-PCR. Pluripotent (Oct-4, SSEA-3, SSEA-4, Tra-1-60 and Tra-1-81) and germ cell markers (VASA, c-KIT, GDF-9, DAZL and ZP4) were immunolocalized in initial scraped OSE cells and 3 week human cultures respectively. Epithelial-mesenchymal transition (EMT) was observed in 3 week OSE cultures and confirmed by RT-PCR studies, that demonstrated downregulation of epithelial markers (cytokeratin 18 and E-cadherin) ,upregulation of mesenchymal marker (vimentin) and transcription factors (Snail and Slug) in human cultures.

Postnatal oogenesis appears to be an evolutionarily conserved phenomenon in mammals. Results suggest that totipotent stem cells in OSE may differentiate into oocyte-like structures whereas mesenchymal fibroblasts generated by EMT may result in supporting granulosa cells. Presence of alkaline phosphatase positive ES-like colonies is being reported for the first time from adult human ovary, similar to those observed recently in adult human testis. Results will help evolve newer strategies to treat infertility and provide a novel source of pluripotent stem cells for regenerative medicine in future. It is tempting to speculate that primordial germ cells persist into adulthood and are currently being detected as VSELs.

EXISTENCE OF OCT-4 PLURIPOTENCY NETWORK IN ADULT HUMAN TESTIS

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Well characterized embryonic stem cell-like colonies have been successfully derived from adult human testicular tissue recently. However, the exact cell source that gives rise to these colonies has not been identified. Dedifferentiation of spermatogonial stem cells (SSCs) has been postulated to be the probable underlying mechanism.

Present study was undertaken to evaluate the localization and expression of pluripotent markers in human testicular tissue obtained from men undergoing orchidectomy for prostate cancer, by various cell and molecular based techniques. Pluripotent markers Oct-4, Nanog and TERT were detected by RT-PCR. The two Oct-4 transcripts Oct-4A and Oct-4B were identified by Real Time PCR and Western Blot analysis, with 3-5 fold higher expression of Oct-4B. Immunolocalization studies on germ cell smears and paraffin sections using polyclonal antibody, demonstrated the presence of cytoplasmic Oct-4B in A_{dark} SSCs that eventually disappeared in more mature germ cells. A_{dark} SSCs were visualized in chains and pairs, with distinct cytoplasmic bridges which also stained positive for Oct-4. In addition, a novel population of 5-10 um size cells with distinctive nuclear Oct-4A staining surrounded by a thin rim of unstained cytoplasm was detected. Spermatogonial stem cells and spermatids stained positive using anti-5-methylcytosine antibody demonstrating extensive epigenetic changes by DNA methylation in these cell types.

Results provide insight into germ cell proliferation during human spermatogenesis and challenge the existing paradigm. Presence of Oct-4A positive pluripotent stem cells are being reported for the first time in adult human testis and are probably the very small embryonic-like stem cells (VSELs) – probably the left over primordial germ cells. It is hypothesized that these pluripotent stem cells probably undergo asymmetric cell division resulting in A_{dark} SSCs with cytoplasmic Oct-4B. These cells undergo proliferation and initiate lineage-specific differentiation into more committed germ cells. The dark nuclei in A_{dark} SSCs possibly reveal extensive nuclear reprogramming by epigenetic changes when a pluripotent stem cell becomes committed. Besides maintaining normal testicular homeostasis, these pluripotent stem cells may also be implicated in germ cell tumors and result in ES-like colonies. Present study is the first *in vivo* demonstration of pluripotency network in adult human tissue similar to embryonic and induced pluripotent stem cells *in vitro* and may help evolve better strategies to treat infertility, address oncofertility and for regenerative medicine in future.

PRESERVING A TOTIPOTENT GERM-LINE: MECHANISMS UNDERLYING GENOMIC REPROGRAMMING DURING *C. ELEGANS* PRIMORDIAL GERM CELL SPECIFICATION

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Across species, primordial germ cells (PGCs) are insulated from differentiation signals that would otherwise engage somatic gene regulatory cascades and instead preserve their totipotency by maintaining transcriptional quiescence (Seydoux and Braun, 2006). However, molecular mechanisms that enforce near total transcriptional inactivity remain incompletely understood. In *C.elegans* germline blastomeres, global repression of RNA polymerase II is initially mediated by the maternal factor PIE-1. Upon PIE-1's degradation in the newly-born PGCs, chromatin-based mechanisms involving genome-wide erasure of marks of "active" chromatin sustain transcriptional dormancy. In particular, the euchromatic mark, histone H3 dimethylated on lysine 4 (H3K4me2), is removed shortly after the birth of the two PGCs, Z2 and Z3. Mutants that accumulate this mark in PGCs over several generations are sterile (Katz et al, 2009), underscoring the importance of this erasure event in maintaining an immortal germline. Utilizing knockdown techniques, live imaging and biochemical approaches, we are presently attempting to obtain mechanistic insight into this erasure event. Intriguingly, we do not detect an immediate requirement for demethylase activity in the removal of this mark either by RNA interference or in mutant analyses. Therefore, we hypothesized that active histone replacement drives an exchange of modified histones in PGC chromatin for unmodified ones. Consistent with this idea, real time quantitative fluorescence imaging shows increased histone H3 dynamics in nascent PGCs. Further, our results indicate non-redundant requirements for a histone chaperone and components of the SWI/SNF chromatin remodeling complex in removal of the H3K4me2 mark in Z2 and Z3. Ongoing experiments seek to identify biochemical mechanisms underlying the specific regulation/activation of these ubiquitously expressed effectors in Z2 and Z3. Interestingly, transcriptionally competent RNA polymerase II is transiently detected in the PGCs concomitant with H3K4me2 erasure. This observation suggests that active histone replacement could be dependent on abortive rounds of transcription, a theory we are currently testing. Ultimately, the implications of our findings will be discussed in light of the regulatory logic that underlies germ cell specification and safeguards its totipotency.

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MOUSE MAELSTROM PROTEIN, A PIRNA PATHWAY COMPONENT, FORMS LARGE MRNP COMPLEXES DURING SPERMATOGENESIS

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Mouse Maelstrom (MAEL) protein is essential for piRNA-mediated transposon silencing during embryonic and post-natal germ cell development. Disruption of Mael results in delayed DNA methylation of transposable elements in fetal prospermatogonia followed by spermatocyte arrest and apoptosis during meiotic prophase I. Arrested spermatocytes exhibit massive failure of synapsis of homologous chromosomes and derepression of transposon silencing in zygonema.

To begin to understand the role of MAEL in spermatogenesis from the biochemical perspective, we wanted to characterize MAEL protein and MAEL-Containing Complexes (MCCs). We have taken two approaches in identifying MCCs: 1) fractionation experiments to characterize stable MCCs and 2) immunoprecipitation of MAEL to identify associated proteins and RNA. Size exclusion chromatography and velocity sedimentation have shown that MAEL forms large RNA-dependent complexes in adult testes. Further studies are underway to identify the components (RNA and proteins) of these fractionated MCCs.

MAEL immunoprecipitation studies from unfractionated testes lysates have failed to detect MCCs containing piRNAs; however, identified associated proteins include a number of RNA-binding proteins such as cold shock domain proteins (MSY1, 2, & 4) and Processing body components (DCP1a & EDC4). Current studies will determine whether MCCs associate with longer RNAs. MAEL immunoprecipitation has also revealed a consistent association between MAEL and TDRD6, a tudor domain containing protein that is essential for spermiogenesis. Immunofluorescence has confirmed that a MAEL-TDRD6 association, suggesting that these two proteins may form a complex during late spermatogenesis. The nature of the association between MAEL and TDRD6 is currently being investigated and we will present the results of these studies.

THE ROLE OF DNMT3L IN MAINTAINING SPERMATOGONIAL STEM CELLS' PROPERTY DURING MOUSE GERM CELL DEVELOPMENT

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Epigenetic modifications have been discovered to be one of the most important regulatory mechanisms for controlling the fate of stem cells. Among the many classes of stem cells, germline stem cells attract much attention due to its unique property in storing and producing cells that transit the genome from one generation to another. DNA methytransferase 3-like (Dnmt3L) is a key epigenetic modifier in maintaining male germ cell development by silencing retrotransposons. Dnmt3L also recruits other epigenetic modifiers, including Dnmt3a, Dnmt3b and HDAC1. Due to Dnmt3L's essential epigenetic properties, questions have been raised about whether it participates in the mysterious regulating pathway for spermatogonial stem cells (SSC), which need epigenetic silencing machinery to keep it quiescent for a sufficient period in order to accumulate enough epigenetic information to support fully functional germ line development.

Here we discovered Dnmt3L's epigenetic role in controlling SSC property. Dnmt3L-/- and control mice testes from 4 to 13 days postpartum were used to demonstrate the initiation of SSC formation to the onset of meiosis in mice, respectively. An apparent reduction and change of expression location for germ cell markers such as *Vasa* and *Stra8* were found in Dnmt3L-/- testis, indicating Dnmt3L's participation in the overall germ cell property in neonatal testes. We also observed a reduction in expression of *Plzf*, an SSC marker and zink-finger protein that is essential for SSC maintenance. *Plzf* is also an epigenetic modifier for stem cells that recruits polycomb group proteins and HDAC. In previous studies, the absence of *Plzf* also affects the perinuclear staining of tri-methylated histone H3 lysine9 (H3K9) and histone H4 lysine20 (H4K20) in the testis, which are indications of the quiescent status of SSCs. In our study, the sub-cellular localization and the expression level of HDAC seems to be affected in the Dnmt3L-/- germ cells, associated with the loss of perinuclear staining of trimethylated H3K9 and H4K20. These data indicate that Dnmt3L is part of the epigenetic control mechanisms in keeping the SSCs quiescent. Our findings have made a novel link between Dnmt3L and stem cell property control in the mammalian germ line.
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HIGH RESOLUTION TRANSCRIPTOME ANALYSIS OF *DROSOPHILA* MALE GERMLINE STEM CELL LINEAGE

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To effectively utilize stem cells in regenerative medicine, the molecular mechanisms underlying their maintenance and proper differentiation must be thoroughly understood. To achieve this goal, we are using a systematic approach to analyze transcription profiles of normally developing germ cells at discrete but continuous differentiation stages. We have developed a strategy to isolate a single cyst of germ cells (encapsulated by two somatic cyst cells) from *wild-type* testes of *Drosophila*. We have successfully used these single germ cell cysts as the starting material to profile the transcriptome using RNA-seq technology. Together, the data from every stage germ cell cysts delineates a high resolution transcription profile in the entire germline lineage. Based on analyses of these data, we have obtained several exciting findings: (1) We found that the immediate daughter cell (i.e. gonialblast) derived from germline stem cell has a significantly higher level of multiple chromatin regulators. We are in the process of investigating the *in vivo* functions of these chromatin factors in regulating germ cell differentiation. We are also interested in the mechanisms that germline stem cells use to keep these chromatin factors at a low level, in order to maintain their identities. (2) We found that the transit-amplifying cells, known as spermatogonia, share a high degree of similarities in their transcriptomes. (3) We confirmed that there is a dramatic transcriptome switch from mitotic spermatogonia to meiotic spermatocyte, consistent with the conclusions based on previous microarray and RNA-seq studies using the entire testes. (4) We also found that there is a substantial transcriptome change from early to late spermatocyte, suggesting that the maturation of spermatocyte involves changes in transcription at the global level. We propose that the early spermatocyte-enriched genes set up the chromatin status to turn on a transcription wave for terminal differentiation genes, which accumulate as late spermatocyte-enriched genes. In summary, our single cyst-resolution transcriptome analyses reveal many interesting features of this stem cell lineage. We will discuss these unpublished data and potential applications of them.

IDENTIFICATION AND CHARACTERIZATION OF GENES INVOLVED IN SPERMATOGENESIS IN THE PLANARIAN *SCHMIDTEA MEDITERRANEA*

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There are two strains of the freshwater planarian, *Schmidtea mediterranea*: the sexual strain is a cross-fertilizing hermaphrodite with reproductive organs that develop post-embryonically; the asexual strain reproduces by transverse fission and does not develop reproductive organs. Sexual planarians have numerous testes that are easily visualized and can be regenerated de novo. This interesting biology, combined with recently developed molecular, cellular, and genomic tools make the planarian an attractive model organism in which to identify and characterize genes involved in spermatogenesis. The formation of sperm involves many conserved pathways and mechanisms that are not well understood. To identify genes expressed differentially during different stages of spermatogenesis, we utilized microarrays comparing gene expression in sexual versus asexual planarians. This analysis identified >800 genes that were upregulated in sexual animals. An in situ hybridization screen was then performed on the subset of genes whose orthologs have been implicated in transcription, signal transduction, and the cytoskeleton, as well as a group of genes that are novel but evolutionarily conserved. Expression patterns in the sexual animals show that 82% of these genes are expressed in the reproductive system. Based on these results, we used bacterially expressed double stranded RNA to knock down genes expressed in the planarian testes. From this ongoing RNA interference screen, we have identified several genes required for proper spermatogenesis. Here we describe the phenotypic characterization of these RNA knock downs. This work provides a platform for better understanding species-specific genes, as well as genes that are conserved among organisms, that regulate spermatogenesis.

A LINK BETWEEN CHROMATIN SILENCING AND OOCYTE LOSS DURING MEIOSIS

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During prophase I of meiosis, maternal and paternal homologous chromosomes synapse and recombine. In humans and mice, defects in synapsis can lead to reproductive consequences, such as impaired fertility, spontaneous abortions and developmental disorders, such as Down syndrome. Meiotic silencing of unsynapsed chromatin (MSUC) is an immediate response to asynapsis and leads to the transcriptional repression of genes associated with unsynapsed chromosomes at the pachytene stage of meiosis. In males, MSUC is required for normal fertility by silencing the genes on the heteromorphic X and Y chromosomes, which remain predominately unsynapsed at pachytene. However, it is unclear how meiotic silencing can affect the oocyte. Since the XX oocyte does not normally trigger MSUC, we examined the consequence of silencing on oocytes containing unsynapsed chromosomes. We first studied XO female mice, which have a single X chromosome and a shortened reproductive lifespan owing to excess oocyte loss occurring during prophase I. We hypothesized that silencing of the unsynapsed X chromosome could contribute to XO germ cell loss by inactivating developmentally-critical genes on the X chromosome. Indeed, we found that XO oocytes with a silenced X chromosome were preferentially eliminated during prophase I. By contrast, we did not observe an oocyte loss associated with silencing in oocytes containing accessory chromosomes (human chromosome 21 or mouse chromosome Y). This was expected since silencing of the non-essential genes on these accessory chromosomes should be tolerated in the oocyte. We then examined the transcriptional activity of genes on unsynapsed chromosomes in oocytes using gene-specific RNA FISH. Unlike in the male germ line, in which silencing of the X and Y chromosomes is complete, in the female germ line we found that silencing is incomplete; some genes escape silencing in a proportion of oocytes. Together, these observations indicate that meiotic silencing is associated with XO oocyte loss and impaired fertility, and that the severity of this loss may depend upon which genes are inactivated within individual oocyte.

OCT-4 EXPRESSION DURING PORCINE GERM LINE DEVELOPMENT

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The germ line is established in animal embryos with the formation of primordial germ cells (PGC), which give rise to gametes. A critical requirement of the germ cell precursors is to maintain pluripotency and prevent differentiation into somatic cells. In mice, germ cell precursors are transitorily segregated from the embryonic domain to the base of the allantois before initiating their migration to colonize their definitive location in the future gonad. Expression of the pluripotency gene *Oct-4* (also known as Pou5f1) and which identifies epiblast cells gradually disappears from differentiating somatic cells after gastrulation, but remains expressed in nascent germ cell precursors. Expression of Oct-4 during mouse germ cell specification is critical for germ cell survival.
The aim of this work was to study the expression of *OCT-4* in developing pig embryos to determine whether this marker could be used as a method to track PGC precursors.
OCT-4 RNA was abundantly expressed in epiblast and early primitive streak stage embryos. Late primitive streak embryos showed marked reduction in OCT-4 in the area of the streak and were maintained in the anterior ectoderm. They then became restricted to a small cluster of cells posterior to the primitive streak. This cluster was later localized in the extraembryonic tissues in neural groove and somite stage embryos (between 3-8 somites). Immunostaining detected OCT-4 protein in presumptive PGC as scattered dots in the posterior end of early gastrulating embryos, and in slightly more advanced stages OCT-4 positive cells appeared in distinctive extraembryonic clusters. Clear OCT-4 staining was also detected in single migratory PGC (8-26 somite embryos) before arriving at the genital ridges. Additional SSEA-1 staining confirmed the germ cell identity of the OCT-4 cells during these stages.
In conclusion, these results demonstrate that OCT-4 can be used to track early germ cell precursors in porcine embryos and is ideally suited for analyzing expression of other early germ cell markers. We will present preliminary findings on the expression profile of BLIMP-1 in early pig embryos.
(Project and DA Contreras were funded by Conacyt-Mexico).

ANALYSIS OF *DROSOPHILA MELANOGASTER* TUDOR: LOGIC OF A DYNASTY

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Tudor (Tud) is a large protein of 2515 amino acids consisting of 11 tudor domains that bind proteins that contain symmetrically dimethylated Arginines (sDMAs). Tud is maternally expressed in the *Drosophila melanogaster* embryo, and required for germ cell formation, it is the most downstream of "grandlchild-less" genes that control the assembly of the germ plasm. Tud is thought to scaffold the germ plasm in the posterior embryo and was recently found to bind Aubergine (Aub), a Piwi protein, in an sDMA dependant manner. A study from our lab showed that the last five C terminal Tud domains are sufficient for germ cell formation. We are studying the role of Tud in germ cell formation, using three approaches: investigate which specific Tud domains are required for its interaction with Aub; since Tud mutations have a less severe phenotype than other mutations in grandchild-less genes, verify if Tudor could be redundant with another tudor domain containing protein, and address whether Tud localization depends on its interaction with the Csul/Vls complex, which catalyzes Arginine symmetrical dimethylation.

THE *C. ELEGANS* ADULT MALE GERMLINE: STEM CELLS AND SEXUAL DIMORPHISM

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The hermaphrodite germline of *Caenorhabditis elegans* provides a well-defined and extensively studied model for stem cell controls and the sperm/oocyte cell fate decision, but the *C. elegans* male germline has largely been ignored. This work focuses on the mitotic region in adult males. Our goals were to establish a stem cell model that was related to but distinct from the hermaphrodite GSC model and to identify sex-specific features of potential relevance to the sperm/oocyte decision. Our results provide evidence for the existence of adult male GSCs with properties similar to those of hermaphrodite GSCs (lack of cell cycle quiescence, lack of reproducibly oriented divisions). They also demonstrate that the length of the mitotic cell cycle is sex-specific throughout the mitotic region, including germ cells adjacent to the DTC within the niche. We conclude that all germ cells possess sexual identity, defined as the possession of sex-specific traits, and propose that sexual identity is not equivalent to commitment to a sperm or oocyte cell fate.

IDENTIFIED BY RNAi NEW GENES IN THE CONTROL OF GERMLINE PROLIFERATION IN *C. ELEGANS*

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Cell proliferation is a key step in germline development that requires precise spatial and temporal regulation to assure a reproductively competent individual. GLP-1/Notch signaling is important for maintaining a proliferating population of germ cells: loss of *glp-1* activity causes all germ cells to differentiate. We have shown that Notch-independent signaling pathways such as insulin/IGF contribute to the robust proliferation of the larval germ line that is required for optimal fecundity. To identify additional genes and pathways required for robust larval germline proliferation, we performed a genome-scale post-embryonic RNAi modifier screen. Our screen was based on a genetic strategy that allowed us to identify genes required for the developmental control of germline proliferation/differentiation, rather than the many genes required for cell proliferation per se. To improve the throughput of the RNAi screen we performed the screening in liquid culture in 96-well plates and we acquired images of each well with a semi-automatic image-capture system. We identified genes that cover a broad spectrum of families and functions that give us a more extensive knowledge of the control of germline proliferation. Of particular interest, we found a novel dauer-independent role for TGFß signaling to promote germline proliferation.

CHROMATIN REMODELING IN MALE HUMAN SEX CHROMOSOMES DURING MEIOTIC PROPHASE I

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In mouse and human, there are a number of mainly genetic observations that highlight the importance of the sex chromosomes for spermatogenesis. Essential for the progression of meiosis is proper formation of the sex body in which during first meiotic prophase, the partially synapsed X and Y chromosomes form a separate chromatin domain. Subsequently, at a later prophase stage genes on the sex chromosomes are silenced. This process is called meiotic sex chromosome inactivation (MSCI). Our project is focussing on sex chromatin behaviour, including MSCI and comparing fertile men with infertile azoospermic patients. Testicular material from patients with non-obstructive azoospermia and from fertile control men is obtained in the context of assisted reproductive techniques. Using immunofluorescence, RNA and DNA FISH, sex body formation and MSCI are followed.
During MSCI in the mouse and in man, nucleosomes from the X and Y chromosomes, containing histones 3.1 and 3.2 are replaced by nucleosomes with histone 3.3. This process can be used as a marker for sex chromosome silencing*. In a small patient group we studied sex chromosomal nucleosome eviction efficiency by using an antibody that specifically recognizes the histone variants 3.1 and 3.2 but not 3.3. Results show variation within (and between) patients and controls in the degree of nucleosome eviction at X and Y chromatin. In mouse this variation was never observed and nucleosome eviction is always fully completed. Preliminary results using RNA FISH to detect Cot-1 repeat expression confirm that there is variation in MSCI efficiency within patients. Furthermore, we expect to link the degree of H3.1/3.2 nucleosome eviction to the degree of Cot-1 repeat expression. Also, nucleosome eviction, in the mouse, is accompanied by changes in histone 3 and 4 N-tail modifications. Our data indicate this pattern to be reproduced in the human, albeit with a lower accuracy.

*GW van der Heijden, AHAA Derijck, E Posfai, M Giele, P Pelczar, L Ramos, DG Wansink, J van der Vlag, AHFM Peters, P de Boer. Chromosome-wide nucleosome replacement and H3.3 incorporation during mammalian meiotic sex chromosome inactivation. Nature Genetics 2007, 39, 251-258

PAN-1, A P-GRANULE ASSOCIATED NOVEL PROTEIN, IS ESSENTIAL FOR *C. ELEGANS* GERMLINE DEVELOPMENT

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In *Caenorhabditis elegans* P granules are germline-specific RNA-containing granules that segregate into the germline precursor cell during early embryogenesis. Germline RNA helicase-1 (GLH-1) is a constitutive P-granule component important for *C. elegans* fertility. Here PAN-1, identified as a GLH-1 binding partner in yeast two hybrid screening, is confirmed to bind GLH-1 by GST pull-down assays. *pan-1(RNAi)* results in an intriguing early larval arrest phenotype in the progeny of injected mothers; the larval arrest is also seen in the homozygous worms of the deletion strain *pan-1(gk142)*. The novel PAN-1 protein contains thirteen leucine-rich repeat motifs and, in the PAN-1 N-terminus, limited homology to a *C. elegans* F-box domain. Anti-PAN-1 antibodies reveal PAN-1 is germline-enhanced, but not germline-specific. PAN-1 co-localizes with GLH-1 in P granules from the first larval (L1) stage onward in development, but is not localized to embryonic P granules. The localization of PAN-1 to P granules beginning in larval development and the lack of recognized RNA binding motifs distinguishes PAN-1 from most P granule components. *pan-1(RNAi)* into the somatic RNAi-defective strain *rrf-1(pk1417)* rescues the *pan-1* larval arrest phenotype, presumably a somatic PAN-1 function, and knocks-down germline PAN-1. These *pan-1(RNAi) rrf-1* adult progeny are sterile, with variable phenotypes including progeny with very tiny germlines, a phenotype also seen with *glh-1(RNAi)*. By western analysis, the *pan-1(RNAi) rrf-1* adult worms with small germlines have reduced GLH-1 levels. Thus, PAN-1 is essential for *C. elegans* fertility and may play a protective role in regulating GLH-1 homeostasis.

THE MICRORNA PATHWAY REGULATES THE TEMPORAL PATTERN OF NOTCH SIGNALING IN DROSOPHILA FOLLICLE CELLS

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Multicellular development requires correct spatial and temporal regulation of cell division and differentiation. These processes are frequently coordinated by the activities of various cell signaling pathways. Notch signaling represents one of these fundamental pathways through which animals direct important cellular decisions such as cell cycle program and differentiation. In addition to the important roles Notch signaling plays in animal development, misregulation of Notch has also been linked to a number of human diseases, including stroke and cancers such as leukemia and osteosarcoma. From a screen for modifiers of Notch signaling in Drosophila we have identified the RNA helicase Belle, a recently described component of the RNA intereference pathway, as in important regulator of the timing of Notch activity in follicle cells. We find that loss of Belle delays activation of Notch signaling and also delays the subsequent inactivation of the pathway in later development. These effects on Notch signaling result in delayed differentiation and defects in cell cycle. Because mutations in well-characterized microRNA components phenocopy the Notch defects observed in belle mutants, we propose that Belle is functioning in the microRNA pathway in follicle cells. The effect of loss of microRNAs on Notch signaling occurs upstream of Notch cleavage because expression of the constitutively active intracellular domain of Notch in microRNA-defective cells restores proper activation of Notch. Furthermore, we present evidence that the Notch ligand Delta in the follicle cells is likely an important target of microRNA regulation in this process and regulates the timing of Notch activation through cis inhibition of Notch. Here we have uncovered a complex regulatory process in which the microRNA pathway promotes the activation of the Notch pathway, possibly by repressing Delta-mediated inhibition of Notch in the follicle cells. Together these findings describe a novel mechanism in the temporal control of the cell cycle and differentiation.

CHARACTERIZATION OF GERM CELL DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELLS WITH TURNER SYNDROME.

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The second X chromosome is silenced in women in order to compensate dosage. However, there are regions on the X chromosome, the pseudoautosomal regions along with specific loci across the entire chromosome, which are not silenced. Turner syndrome women have complete or partial loss of their second sex chromosome, leading to a 45,X karyotype. The majority of Turner syndrome women are infertile because of oocyte loss resulting in “streak” ovaries, which lack follicles. In contrast to humans, XO mice are fertile and phenotypically normal, thus they can not be used as a model organism to study Turner syndrome. I have generated and characterized induced pluripotent stem cells (iPSCs) from Turner syndrome and Premature Ovarian Failure (POF) patients, which can be used to study the role of the X chromosome in the formation and differentiation of the human female germ cell lineage.
A Turner syndrome fibroblast line, fibroblasts from a POF female and control female were reprogrammed using two sequential retroviral transductions of the 4 Yamanaka factors (OCT4, SOX2, KLF4, cMYC). The Turner iPSC clones exhibited a 45, X karyotype, while the POF and control iPSC clones maintained a normal 46, XX karyotype. All clones from the three lines expressed the key plruipotency markers. All clones from each line were injected subcutaneously into female severe combined immunodeficient mice, which developed teratomas with tissues characteristic of all three germ layers. In vitro differentiation was performed by spontaneous differentiation of embryoid bodies until beating cardiomyocytes were observed. Differentiated cells were positive for cells of the three germ layers. To optimize iPSC line generation, I have also used a lentiviral vector carrying all four Yamanaka factors in one single excisable vector. Turner primary fibroblasts, a Turner cell line, control and POF fibroblasts were reprogrammed using the lentiviral system and either physiological or atmospheric oxygen concentrations. These colonies are currently being characterized for karyotype, pluripotency marker status, and their ability to differentiate in vitro and in vivo. These cells will then be treated with Cre recombinase to excise out the single vector integration and pluripotency status will again be examined to show complete reprogramming. These iPSC lines will then be differentiated to the germ cell lineage to address the role of haploinsufficiency of the X chromosome in human germ cell lineage differentiation. The overall goal of these studies will form the foundation for clinical applications to preserve and/or restore fertility in women with ovarian failure.

MOLECULAR AND GENETIC DISSECTION OF THE T-STAR RNA BINDING PROTEIN IN MOUSE TESTIS.

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The RNA binding protein T-STAR is highly expressed in the testis within the nuclei of mouse spermatocytes and round spermatids, and is a member of the STAR family which includes Sam68 and SLM1. The global functions of T-STAR are largely unknown, but Sam68 is essential for fertility and controls translation during spermiogenesis. T-STAR protein shows a closely overlapping pattern of expression in mouse germ cell development to Sam68, but SLM1 is not expressed in the testis. To explore the function of mouse T star protein we have carried out high resolution mapping of binding sites within the mouse germ cell transcriptome, and generated a conditional mouse model in which exon 2 can be removed by Cre-Lox recombination. Western blot and immunohistochemical analysis of testis tissue from the conditional mouse model after ubiquitous Cre recombinase expression driven by the PGK promoter demonstrated that we have created a null allele of T-star. However, unlike the Sam68 knockout mouse which is infertile, both homozygous male and female T star knockout mice are fertile and have no problems feeding their young. Furthermore, examination of the seminiferous tubules demonstrated no morphological defect in the knockout mice. Mutant mice contained wild type levels of epididymal sperm with normal morphology which are capable of undergoing the acrosome reaction. To identify the physiological targets of T-STAR in the mouse testis we carried out HITS-CLIP, and identified 100,000 binding sites within the mouse testis transcriptome including some within or nearby alternatively spliced exons. We are using ectopic assays utilising minigenes, and analysing mouse testis RNA to find out if T-STAR is largely redundant with Sam68 in splicing control in the mouse testis, consistent with their overlapping patterns of expression.

INVESTIGATIONS INTO THE MECHANISM AND FUNCTION OF MEIOTIC SILENCING IN C. ELEGANS GERMLINE

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Meiotic silencing (MS) is an evolutionary conserved process directing the acquisition of repressive chromatin architecture by asynapsed axes of chromosomes in prophase I of meiosis. Although MS is implicated in elimination of defective meiocytes and reduction of fertility, the direct evidence for its effects on meiotic progression is still missing. We are using *C. elegans* to study the signals that trigger and control MS. Accumulation of H3K9me2, a repressive histone modification, is a hallmark of the MS response in C. elegans. We aim to understand the regulation of this accumulation and the consequences of the H3K9me2 that we observe. For example, the appearance of H3K9me2 could be used as a signal for meiotic defects, yet quantitative analysis of the level of apoptosis showed that elevated apoptotic response in the germline carrying nuclei with unpaired chromosomes could not be attributed to changes in H3K9me2 levels. By examining levels of H3K9me2 on meiotic chromosomes of many meiosis mutants, including those defective for loading of cohesins, pairing center proteins or components of synaptonemal complex, we are investigating whether proper organization of axial core of unpaired chromosomes is necessary for MS signals. An updated model for MS mechanisms, resulting from our studies on the targets of MS, and the role of MS-dependent H3K9 methylation on transcriptional repression during meiosis, will be presented.

MODELLING SPERMATOGENESIS IN VITRO USING MOUSE EMBRYONIC STEM CELLS

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One of the essential bases for treatment of male infertility is the understanding of mammalian spermatogenesis. This process comprises a remarkable program of cell differentiation, migration and dramatic changes in cell morphology, epigenetic status and gene expression. However, due to the complexity of the sophisticated organization of the seminiferous epithelium and the difficulty in isolating specific spermatogenic cell types the study of spermatogenesis has been held back. The lack of established cell lines that are able to recapitulate any of the multiple differentiation steps of the spermatogenesis program in vitro, where experimental manipulation is possible, makes things even more complicated.
All germ cell types analyzed to date (including mature germ cells) can be identified by marker analysis during ES cell differentiation. Recent reports where the generation of post-meiotic germ cells from mouse embryonic stem (ES) cells were described, present a hope that directed differentiation of ES cells into germ cells can potentially provide a robust cell-based model for investigating the molecular events that regulate germ cell development. For these purposes we developed three stable transfected mouse ES cell lines (R1) where each one harboured stage-specific fluorescent marker gene constructs. The expression of the fluorescent genes was driven by germ cell specific promoters active at different spermatogenic stages in vivo. Differentiation of ES cells using a germ cell differentiation protocol with defined factors allowed the isolation of pre-meiotic, meiotic and post-meiotic germ cells populations by FACS. The gene expression profile of ES-derived germ cells was analysed by RT-PCR and compared with undifferentiated ES cells. Differentiated cells expressed a number of germ cell specific genes and proteins.
We propose that differentiation of ES cells to the germ cell lineage could facilitate analyses of the mechanisms underlying spermatogenesis. This model will provide a readily accessible system for isolation of specific type germ cells, for examining changes in the methylation pattern, and for the first time will allow a directed effort to uncover critical pathways that direct germ cell specification and differentiation.

BLANKS, A NUCLEAR SIRNP COMPONENT, IS REQUIRED FOR DROSOPHILA SPERMIOGENESIS

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Small RNAs and a diverse array of protein partners control gene expression in eukaryotes through a variety of mechanisms. By combining short interfering RNA (siRNA) affinity chromatography and mass spectrometry, we have identified the novel double-stranded RNA-binding domain (dsRBD) protein Blanks as an siRNA-associated protein from Drosophila S2 cells. We find that Blanks is a nuclear factor that contributes to the efficiency of RNAi. Biochemical fractionation of a Blanks-containing complex shows that the Blanks siRNP is unlike previously described RNA-induced silencing complexes and associates with the DEAD-box helicase RM62, a protein previously implicated in RNA silencing. Deep sequencing of Blanks-associated small RNAs from S2 cells reveals enrichment in ~21-nucleotide RNAs derived from transposable elements. In flies, Blanks is highly expressed in testes tissues and is necessary for post-meiotic spermiogenesis, but loss of Blanks is not accompanied by detectable transposon derepression. Instead, genes related to innate immunity pathways are upregulated in blanks mutant testes. These results reveal Blanks as a novel component of a nuclear siRNP that contributes to essential RNA silencing pathways in the male germline.

DAZL-DEPENDENT LICENSING OF GAMETOGENESIS: THE GATEWAY TO SEXUAL DIFFERENTIATION OF FETAL GERM CELLS

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Oocytes and spermatozoa derive from a fetal cell type shared by the sexes: the primordial germ cell (PGC). The resultant PGCs migrate to the developing somatic gonad, where they ultimately give rise to either oocytes or spermatozoa. These opposing sexual fates are determined not by the PGCs' own sex chromosome constitution (XX or XY), but by the sexual identity of the fetal gonad to which they migrate. How unisex PGCs acquire the ability to differentiate sexually is unknown. We have discovered a pivotal role for DAZL, an RNA-binding protein expressed in both ovarian and testicular germ cells. Germ cells in C57BL/6 *Dazl*-deficient fetuses – whether XX or XY – show no demonstrable features of either male or female differentiation. Instead, they retain a sexually undifferentiated state similar to that of migrating PGCs. We propose that PGCs of both XX and XY fetuses undergo an active, *Dazl*-dependent developmental transition, which we term licensing, that enables the resultant gametogenesis-competent cells to respond to feminizing or masculinizing cues produced by the fetal ovary or testis, and hence to embark on oogenesis or spermatogenesis.

IN VIVO AND IN VITRO HUMAN MALE GERM CELL DIFFERENTIATION

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Generation of germ cells from human pluripotent stem cells is a critical goal that will facilitate our understanding of how the germ line differentiates during fetal life. Human male primordial germ cells (PGCs) can be temporally categorized into the following developmental steps: i) Specification and migration (week 2-4), ii) Gonadal colonization (week 4-6), iii) Sex determination (week 6-7) and iv) Quiescence (week 18-20). In the current study we used human embryonic stem cells (hESCs) to induce PGC differentiation in order to determine the transcriptional program of newly specified human PGCs. We also used human male fetal gonads from developmental week 7-18 to examine endogenous human PGC development following gonadal colonization. Human male fetal gonads at week 7-8 uniformly express OCT4, VASA, cKIT and SSEA1. We find that SSEA1 is not restricted to PGCs during this period, and is no longer enriched on the germ line after 10 weeks. Using histone H3K27me3 we show that human PGCs undergo genome wide reprogramming between week 8-14 resulting in a complete loss of H3K27me3. To understand the transcriptional program of post-reprogrammed human PGCs we performed FACS for cKIT on week 14-15 human gonads. cKIT bright cells are enriched in known germ cell genes including NANOS2, NANOS3, DAZL, VASA, OCT4 and cKIT relative to the non-germ cells (SSEA1 sorted population). Using real time PCR we verified the microarray, and analyzed a number of candidate genes in cKIT sorted germ cells from week 13-18 and compared this expression to undifferentiated hESCs. Transcription of many imprinted genes is significantly up-regulated relative to hESCs from week 13 onwards. At week 15 we observed a >5 fold increase in NANOS2, VASA, DPPA3 and DAZL transcripts and a pulse in transcription of the meiotic marker SYCP3. To acquire the transcriptional program of pre-gonadal PGCs we used an OCT4-GFP reporter hESC line created by homologous recombination. Using either adherent or EB differentiation, we isolated the cKIT/OCT4 double positive population by FACS and performed microarray. Comparing the transcriptional program of cKIT/OCT4 in vitro PGCs (iPGCs) to cKIT positive 14-15 week endogenous PGCs, and hESCs showed that the iPGCs clustered with the endogenous PGCs. By Real time PCR we confirmed that the iPGCs up regulate BLIMP1 and PRDM14, however similar to week 13 cKIT positive endogenous PGCs, VASA, DAZL or DPPA3 are not enriched in this population. Further experiments will verify the methylation changes that occur in newly specified PGCs in vitro and also during re-programming in vivo.

GENERATION OF INDUCED PLURIPOTENT STEM CELL LINES FROM PATIENTS WITH SPORADIC FORM OF PARKINSON'S DISEASE TO UNDERSTAND REPROGRAMMING

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Human embryo development begins with the reprogramming of the germ cell pronuclei, a series of cleavage divisions and the activation of the embryonic genome on Day 3, following a period of transcriptional silence. We are exploring the hypothesis that errors in reprogramming and early embryo development may be propagated ultimately as sporadic disease. To address this hypothesis, we have focused on exploration of sporadic Parkinson's disease, as it is a cell autonomous (at least in part), resulting from loss of dopaminergic neurons in the midbrain. Here we report the generation and characterization of disease specific induced pluripotent stem cell (iPSC) lines using a traditional retroviral system and a new excisable lentiviral system to study reprogramming of adult dermal fibroblasts. After extensive characterization we have shown that these iPSC lines have a normal karyotype, express pluripotency markers and were able to differentiate into three germ layers both in vitro and in vivo. Furthermore, we could directly differentiate these cells into dopaminergic neurons and observe a phenotype that may be relevant to the generation of sporadic disease. Our findings suggest that these iPSC lines derived may be useful as a platform for investigative research in modeling human sporadic PD and understanding reprogramming and epigenetic errors in human embryology.

ACTIVE MIGRATION OF THE GFRα1-EXPRESSING ASINGLE SPERMATOGONIA IN MOUSE SEMINIFEROUS TUBULES

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Our recent study implies that the population of GFRα1+ Asingle (As) cells is the best related to the actual stem cells that support normal steady-state spermatogenesis in adult mouse testis. However, the behavior of GFRa1+ spermatogonia has not directly been observed. One of the biggest questions raised here is whether GFRα1+ As cells are immobile and stay at specific position like the germline stem cells in Drosophila testis and ovary. To approach this, we have performed live-imaging analyses of mouse testis harboring GFRα1-expressing cells labeled with GFP. Interestingly, it has been revealed that GFRα1+ As spermatogonia are actively moving: some are migrating over the seminiferous tubules, while others are screaming along the blood vessels. This is an anatomically and physically surprising phenomenon because GFRα1+ spermatogonia are closely surrounded by Sertoli cells and differentiating spermatogonia on the basement membrane of seminiferous tubules. Active migration of GFRα1+ As suggests that mouse spermatogenic stem cells may not be tethered to a definitive microenvironmental niche in contrast to Drosophila germline stem cells, although the possibility that there exists a small population of GFRα1+ cells which are anchored to specific cites and act as actual stem cells cannot be excluded.

MINICHROMOSOME MAINTENANCE 9(MCM9) HAS A ROLE IN THE GERM CELL POOL, SPERMATOGONIAL STEM CELL MAINTENANCE, AND GENOME STABILITY.

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Minichromosome maintenance (MCM) proteins help ensure that the genome replicates only once per cell cycle. Six of these proteins, MCM2,3,4,5,6 & 7, comprise the replicative helicase involved in initiation and elongation of the DNA replication fork. These proteins contain a highly conserved domain, the MCM domain, which is conserved throughout eukaryotes. This domain is ~200 amino acids long and contains Walker A and Walker B motifs, which are involved in ATP binding and ATPase activity. *Mcm9* is widely expressed and contains the full MCM domain with a non-canonical Walker B motif. Additionally, it generates a shorter splice isoform encoding a truncated protein lacking the Walker B motif and C-terminus. Recent studies show that MCM9 is a positive regulator of CDT1 and helps load the MCM2-7 complex onto the replication origin (Lutzmann et all 2007). In order to elucidate the function of *Mcm9*, we imported a collection of mouse ES cell lines containing gene-trap insertions within this gene. One of these gene-traps, $Mcm9^{XE518}$, creates a double null, not only disrupting *Mcm9*, but also deletes *Asf1a*, a histone chaperone that is in *Mcm9*'s 7th intron. This allele is mid-gestation embryonic lethal, the cause of the lethality in this allele is unknown, yet *Mcm9* is not essential for early embryonic development. Another gene-trap, $Mcm9^{XG743}$, inserts downstream of the Walker A motif and truncates the full-length protein to the shorter isoform. Homozygotes are viable, however there is some wild-type isoform expressed at a low level. These animals have germ cell depletion, and in males it is progressive with age. Mutant testes contained seminiferous tubules displaying any of 3 types of abnormalities: 1) a lack of germ cells; 2) meiotic arrest; and 3) absence of spermatogonial cohorts in the presence of meiotic and postmeiotic waves. These mice were aged to 1yr and mutant males primarily developed liver tumors. These data show that Mcm9 is not essential for early DNA replication. However, Mcm9 has a role in genome stability, a role in the germ cell population, during meiosis, and in spermatogonial stem cell maintenance.

PERIODIC ACTIVATION OF RETINOIC ACID SIGNALING CREATES STAGE-DEPENDENT GENE EXPRESSION IN SERTOLI CELLS DURING MOUSE SPERMATOGENESIS

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Sertoli cells are unique somatic cells in seminiferous tubules and have pivotal roles in the continuous differentiation of male germ cells during spermatogenesis. It has been known that Sertoli cells change their gene expression coincident with the alteration of interacting germ cell groups that can be categorized into twelve stages (stage I-XII). This indicates that Sertoli cells may provide stage-specific microenvironments that are suitable for spermatogenic cells in each stage. It has been reported that retinoic acid (RA) is critical for the progression of spermatogenesis and RARα, a major receptor for RA in Sertoli cells, is essential for the cyclicity in Sertoli cells. However, it remains unclear how RA signaling regulates the cyclicity in Sertoli cells and what role the cyclicity of Sertoli cells has. In the current study, we first identified stage-dependent genes in Sertoli cells with comprehensive microarray analysis, and classified them to four groups according to the peak expression pattern. Interestingly, the expressions of those genes showing peak during stage I-VI were suppressed, while those showing peak in VII-XII were activated by RA signaling in cultured Sertoli cells. We also found that RA signaling was periodically activated during stage VII-XII using RARE-LacZ reporter mice. These results suggest that the stage-dependent gene expression in Sertoli cells is primarily created by the periodic activation of RA signaling in stage VII-XII. Furthermore, we found that suppression of RA signaling via injecting lentivirus containing dominant-negative form of RARa into adult testes resulted in the disruption of stage-dependent gene expression in Sertoli cells. In addition, we observed a failed recovery of blood-testis-barrier from stage IX during spermatogenesis. Taken together, RA signaling dependent cyclicity in Sertoli cells is essential for the establishment of stage-specific microenvironments and the progression of spermatogenesis.

SEX LETHAL ACTS AUTONOMOUSLY IN THE GERMLINE PROGENITORS TO INITIATE FEMALE DEVELOPMENT IN *DROSOPHILA*

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One of the major decisions that germline cells make during their development is whether to differentiate into eggs or sperms. In *Drosophila*, sexual identity of germline progenitors, or primordial germ cells (PGCs) is regulated both by sex of the surrounding soma and by a cell-autonomous cue. Male sexual development is initiated in the PGCs by the JAK/STAT signal from the gonadal soma. However, it remains elusive how the PGCs initiate female development. It has been widely accepted that *Sex lethal* (*Sxl*) is a sex-determining gene that induce female fate in the somatic tissues, but not in the PGCs. Contrary to the previous view, here we show that *Sxl* act autonomously in the PGCs to induce female development. Its expression is detected in a female-specific manner, and is necessary for feminization of PGCs before they reach gonads. Furthermore, ectopic expression of *Sxl* in male (XY) PGCs is sufficient to induce female fate in these cells, and the resulting PGCs are able to produce functional eggs within female soma. Our data provides strong evidence showing that *Sxl* acts as a master gene that directs female germline fate in sexual development.

THE ROLE OF HEPARAN SULFATE PROTEOGLYCANS IN *DROSOPHILA* GERMLINE STEM CELL NICHE

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Stem cell posses the remarkable characteristics to produce its daughter cell that retain a stem-cell identity and the other that differentiates. Stem cells reside in dedicated cellular microenvironments termed stem-cell niches. These niches dictate a stem-cell identity, maintain the stem cell population, and coordinate proper homeostatic production of differentiated cells. *Drosophila* germ line stem cells (GSCs) are one of the ideal models for stem cell study in vivo. In apical tips of both male and female gonads, GSCs associate with specially differentiated somatic gonadal cells, the niche cells. These niche cells generate niches for GSCs by secreting signaling molecules, such as TGF-beta ligand, Dpp and JAK/ STAT ligand Upd, which are essential for GSC maintenance. Although these facts indicate that region of niche is defined by the spatial distribution of these ligands, molecular mechanisms that regulate ligand distribution within the niche is not characterized.

Heparan Sulfate Proteoglycans (HSPGs) are one kind of glycoprotein which are expressed on the cell surface and/ or in the extracellular matrix. Recent in vivo studies have shown that HSPGs play critical roles in regulating signaling during development by a variety of mechanisms including regulation of extracellular ligand distribution.

Here we show that *Drosophila* HSPGs are essential components of GSC niche. *Drosophila* two glypians, a subfamily of HSPGs, *dally* and *dally-like* were expressed in niche cells of female and male gonads, respectively and were required for GSC maintenance. In GSCs of these glypican mutants, signaling which is essential for GSC maintenance were impaired. Conversely, when dally was ectopically expressed in ovaries, we observed expansion of GSC niche. These results showed that these glypicans define niche region by regulating range of niche signal activity, presumably by controlling ligand distribution. HSPGs are evolutionally conserved molecules, thus our results imply HSPGs are general niche components that define niche region molecularly.

IDENTIFICATION OF THE GERM LINE IN PENAEOIDEAN SHRIMP

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Little is known about the identity and specification of the germ line in penaeoidean shrimp, a major aquaculture species worldwide. A cell of mesendodermal origin has been hypothesized as the primordial germ cell by morphological criteria of large nuclear-cytoplasmic ratio and distinctive chromosomes. Recently, a structure known as the intracellular body (ICB) has been identified in penaeoidean shrimp as a candidate germ granule. The ICB has been found in four species of penaeoidean shrimp (*Penaeus monodon*, *P. japonicus*, *P. vannamei*, and *Sicyonia ingentis*) in vegetal blastomeres from the 2-cell through the 122-cell stage, and is inherited by one of the two mesendoderm cells. The ICB is stained by the nucleic acid stains Sytox Green and SYTO RNASelect, but not Hoechst 33258. Treatment with RNase but not DNase abolishes this staining, indicating that the ICB is enriched in RNA. Ultrastructural examination of mesendoderm cells revealed a mitochondrial cloud adjacent to the ICB and the nucleus, while the ICB contained annulate lammelae. The presence of enriched mitochondria and annulate lammelae provide additional indirect evidence that the ICB lies within the germ plasm in penaeoidean shrimp. Studies are in progress to determine the fate of the ICB in later embryonic stages, trace the formation of the germ line, and identify germ line genes in penaeoidean shrimp. A better understanding of germ line specification in shrimp could allow the development of fertility control technologies for genetic protection of elite lines

THE ROLE OF *DM IME4* IN EGG CHAMBER FORMATION

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Soma-germline interactions are required for proper gamete formation in all multicellular organisms. In S. cerevisiae, a unicellular eukaryote, each individual cell interacts with the environment to determine when to switch from mitotic growth (vegetative asexual state) to meiosis (gametogenesis). IME4 (Inducer of Meiosis 4) is exclusively expressed upon meiotic entry and regulates this mitotic:meiotic switch in yeast. IME4 has highly conserved homologs in D. melanogaster, A. thaliana, M. musculus, H. sapiens, and other higher eukaryotes, but its function in multicellular organisms is presently unknown.

The Drosophila IME4 homolog, Dm ime4, is expressed in ovaries and testes, suggesting an evolutionarily conserved function in gametogenesis. To define the role of Dm ime4, we generated mutations by P-element excision mutagenesis and have exploited transgenic lines in which gene function can be ablated by inducible RNAi. Deletion of this gene or its ablation via RNAi produced the unexpected result that, in contrast to yeast, Dm ime4 is essential for viability. We recovered hypomorphic mutations in Dm ime4 that yield viable adults whose phenotypes reveal critical functions for this gene in gametogenesis. Ovarioles from Dm ime4 mutants consist of fused egg-chambers that contain supernumerary nurse cells with one or several oocytes. Polar cells are absent or mislocalized, and there are no stalk cells. Although this phenotype is complex, the fusion may originate from defective cyst individualization at the posterior end of the germarium. We are currently investigating defects in cyst encapsulation by examining follicle stem cell fates as well as Notch signaling defects in these mutants.

ACTIVATION OF THE GERMLINE TRANSCRIPTIONAL PROGRAM IN DROSOPHILA

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In order to maintain the totipotency of germ cells and prevent differentiation, transcription of zygotic genes must be repressed while transcription of germ genes must be activated. A number of factors have been identified that maintain the undifferentiated state of germ cells by globally inhibiting mRNA transcription. Repressors of RNA polymerase II (RNA pol II) transcription have been well described such as the maternally provided factor, *polar granule component* (*pgc*). However, it is unknown which proteins directly activate transcription in the germ cells after the inhibition of RNA pol II is lifted immediately following gastrulation. Several maternal factors are essential for early pole cell transcription, such as *pgc* and the maternal RNA binding protein Nanos, which represses mitosis, apoptosis, and the somatic differentiation of pole cells. However, neither genetic nor molecular screens have yet identified any maternal factors that encode for transcriptional regulators involved in germline-specific gene expression. Similarly, very few genes have been identified which are transcribed in germ cells at these earliest stages, apart from *vasa*.

This project aims to identify factors involved in regulating germline-specific transcription in early germ cells in *Drosophila* by using the recently developed techniques of RNA-seq (high-throughput sequencing of RNAs) and ribosomal profiling. In addition, utilizing the power of *Drosophila* genetics, these factors will be functionally validated by antisense-RNA screening. Subsequent molecular and genetical analysis of the confirmed factors involved in activating germ gene transcription and repressing somatic transcription will provide much needed insight into the molecular mechanisms that regulate germ cell specification. The proposed research will provide a vast array of data on the transcriptome and 'translatome' of early germ cells, as well as give insights in mechanisms of transcriptional regulation.

REDOX REGULATION OF E-CADHERIN ALTERS DROSOPHILA GERM CELL ADHESION DURING GASTRULATION

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Through a targeted genetic screen for genes that alter Drosophila germ cell migration to the gonad, we identified mutations in the peroxiredoxin gene, jafrac1, that give rise to novel germ cell adhesion defects. During gastrulation, primordial germ cells (PGCs) cluster and associate tightly with the invaginating midgut primordium as they are taken into the embryo; however in embryos from jafrac1 mutant mothers PGC clustering is disrupted, leaving some PGCs outside. We observed similar phenotypes in embryos from DE-cadherin/shotgun (shg) mutant mothers suggesting that this is a DE-cadherin dependent adhesion process. Our further biochemical and genetic evidence strongly suggests that the PGC adhesion defect in embryos from jafrac1 mutant mothers is caused by a hydrogen peroxide-mediated post-transcriptional reduction in DE-cadherin protein levels. jafrac1 is one of three functionally conserved, typical 2-Cys peroxiredoxins in the Drosophila genome. These proteins primarily act as antioxidants by reducing hydrogen peroxide to water; indeed, we show that jafrac1 protects Drosophila from oxidative stress. Orthologs of jafrac1 have been implicated in tumor suppression and growth factor signaling in mammals. Here, we present the first in vivo evidence of a peroxiredoxin regulating DE-cadherin mediated adhesion through hydrogen peroxide. We propose that the initial step of PGC migration in Drosophila is a new genetic model for the analysis of redox-mediated adhesion.

FUNCTION OF TUMOR SUPPRESSOR APC2 AND POLARITY PROTEIN BAZOOKA (PAR3) IN ASYMMETRIC STEM CELL DIVISION AND ITS NOVEL CHECKPOINT IN *DROSOPHILA* MALE GERMLINE STEM CELLS

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Asymmetric cell division is widely utilized by many adult stem cells to balance self-renewal and generating differentiated, short-lived cells, so that tissue homeostasis is maintained throughout life. Mitotic spindle orientation relative to the surrounding tissue is a strategy utilized by many adult stem cells to divide asymmetrically.
Drosophila melanogaster male germline stem cells (GSCs) are a premier model to study stem cell-niche interactions in vivo because of their simple anatomy. Somatic hub cells constitute the GSC niche and provide the fundamental signals required for maintenance of stem cell identity. In the asymmetric division of GSCs, one daughter cell that maintains the contact with the hub retains the stem cell identity, while the other that is positioned away from the hub starts differentiation. This process is achieved by proper centrosome and spindle orientation perpendicular to the hub.
We recently demonstrated that GSCs carrying the misoriented centrosomes do not enter mitosis until their centrosomes are re-oriented, pointing to the presence of a novel checkpoint mechanism that monitors interphase centrosome orientation prior to mitosis (the centrosome orientation checkpoint). Furthermore, we identified Par-1 kinase as a key component of this checkpoint mechanism (Yuan et al.).
In this study, we demonstrate that adherens junctions formed between the hub and GSCs play fundamental roles in orchestrating tumor suppressor Apc2 and polarity protein Bazooka (Par3) in regulation of the GSC centrosome orientation and its checkpoint. Expression of dominant negative cadherin results in delocalization of Apc2 and Bazooka, with concomitant defects in centrosome and spindle orientation. Interestingly, while Bazooka appears to be involved in both the centrosome orientation and its checkpoint, Apc2 is only involved in the centrosome orientation but not checkpoint. We propose that E-cadherin-based adherens junction orchestrates the localization of multiple players involved in the centrosome orientation and its checkpoint.

EXPRESSION OF PLURIPOTENT STEM CELL MARKERS IN GONADAL PRIMORDIAL GERM CELLS OF CHICKEN EMBRYOS

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Embryonic stem cells (cESCs) can be isolated from chick embryos, with the ability to contribute to all somatic lineages in chimaeras, but not to the germ line. However, cell lines (cPGCs) with this ability can be established from embryonic germ cells, but little is known about whether they are pluripotent, whether they express the same molecular markers as cESCs, or whether pluripotency markers are expressed in the parent germ cells while they are in the gonad. Here we examine the expression of the stem cell pluripotency markers SSEA-1, the Oct4 homologue *cPouV* (Lavial et al., 2007), *cNanog*, *cSox2* and *ERNI* in chicken embryonic gonads (HH stage 26-28), cPGCs and cESCs. In cESCs, SSEA-1, *cPouV*, *cNanog*, *cSox2* and *ERNI* are highly expressed but *CVH* (*cVasa*) is not. SSEA-1, *cPouV*, *cNanog* and *ERNI* as well as the germ cell marker, *CVH* (*cVasa*) are expressed in the gonadal ridges and dorsal mesentery, whereas *cSox2* positive cells are only detected in dorsal mesentery. However, the proportion of expressing cells is not always the same for all markers. *CVH*, *cPouV* and *cNanog* are expressed by most cells in the gonad, cPGCs and cESCs. *ERNI* decreases after culture in cPGCs whereas *cSox2* appears to be induced by culture.
In conclusion, some gonocytes express markers of pluripotency and self-renewal even *in vivo*, in their natural gonadal location. This could indicate that these cells are naturally endowed with pluripotency and self-renewal functions, but also suggests heterogeneity and/or dynamic expression of these markers.
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EVOLUTION OF THE GERM LINE-SOMA RELATIONSHIP IN CHORDATE EMBRYOS: A UNIFYING HYPOTHESIS

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The animal kingdom can be divided into two subclasses of species, some which specify primordial germ cells (PGCs) cell-autonomously, by inheriting germ plasm, and others which produce germ cells by induction. Germ plasm has been identified in many species, and in particular those animal models used in many laboratories throughout the world; but phylogenetic evidence indicates that germ plasm is not conserved. Rather, it has been proposed that formation of PGCs by induction, i.e. epigenesis, is conserved (Johnson et al., 2001, 2003; Extavour and Akam, 2003). This raises the question, when most biological mechanisms are highly conserved across vast phylogenetic distances, why should this fundamental biological process have evolved two distinct mechanisms?

Amphibians are uniquely well suited to address this question because anurans (frogs) and urodeles (salamanders) are closely related, though they employ germ plasm and epigenesis, respectively, to specify PGCs in early development. Here we demonstrate the induction of PGCs from axolotl animal caps using molecular markers. The ability of axolotl primitive ectoderm (animal cap) to produce germ cells and somatic cells is the defining characteristic of cells in the pluripotent ground-state, the property exemplified by mammalian embryonic stem cells. We show that the axolotl Nanog gene has conserved activity as a master regulator of pluripotency, demonstrating that pluripotency is conserved from urodeles to mammals. Further, we demonstrate conserved Nanog activity from lower chordates whose embryos produce PGCs by epigenesis, indicating that pluripotency is conserved in the major trunk of chordate evolution. From these data, the absence of Nanog in the genome of Xenopus and teleost fish can only be explained by deletion, and suggests that the evolution of germ plasm makes passage through the pluripotent ground–state expendable.

The repeated emergence of germ plasm in distinct lineages is evidence for a selective advantage conferred on a species by the evolution of predetermined germ cells. Here we present evidence supporting the hypothesis that the evolution of germ plasm alters the ancestral germ line-soma relationship, leading to a more robust mode of embryogenesis. The robustness, however, comes with a cost, since the rapidly evolving species that result are resistant to major changes, whereas pluripotency provides the raw material for the major modifications supporting macro-evolution within the major trunk of the chordate tree.

THE POLYCOMB GROUP GENE *EZH2* AND GERM LINE DEVELOPMENT IN ZEBRAFISH

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Polycomb group (PcG) proteins are transcriptional repressors and play a role in development, stem cell maintenance, and cancer. They exert this function by changing the chromatin structure. Knock-out mice for different PcG proteins often show early embryonic lethality. The progeny of *C. elegans* MES mutants is sterile indicating a role during germ line development. In this study, the role of *enhancer of zeste homolog 2* (*ezh2*), one of the PcG genes, during germ line development of the zebrafish was examined.
Ezh2 mRNA is maternally provided in the zebrafish, indicating this gene is important for early embryonic development. At 72hpf we observed most prominent expression of *ezh2* in the branchial arches, the eyes, the gut, and specific regions of the brain, like the tectum and the mid-hindbrain region. At this time there is no detectable expression of *ezh2* in the primordial germ cells. Zebrafish have no sex chromosomes and around 4 weeks of age sex-determination takes place. Around this time *ezh2* starts to be expressed in the germ line. In adults, *ezh2* is specifically expressed in germ cells, being most prominent in the early stage germ cells.
By TILLing we identified a mutant with a premature stop in *ezh2*, located before the histone methyltransferase (SET) domain. The homozygous stop mutants survive gastrulation and seem to develop normally until the majority dies around day 12. At three weeks of age no homozygous mutants survived. Maternally provided *ezh2* is present in the mutants and probably helps the embryos to survive early embryogenesis. The formation of primordial germ cells is not affected in *ezh2* stop mutants. Since *ezh2* is expressed only after 4 weeks of age in the germ line, this suggests *ezh2* is important for germ line development only at a later time point.
Germ cell transplantations are in progress to obtain zebrafish to study the effect of loss of *ezh2* on germ line development in an otherwise wildtype environment. In addition, we are generating transgenic zebrafish with germ line specific expression of a short hairpin RNA directed against *ezh2*. These two models will provide valuable tools to study the role of *ezh2* during germ line development in vertebrates.

A NOVEL MUTATION RESULTING IN LOSS OF THE GERMLINE IN ZEBRAFISH

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Zebrafish lack obvious sex chromosomes and all individuals experience a stage of juvenile hermaphroditism. Juvenile gonads initiate the expression of both male and female specific genes, such as *anti-müllerian hormone (amh)* and *cytochrome P450 aromatase 19a1a (cyp19a1a)*, respectively. However, by approximately 30 days post fertilization (dpf) presumptive males experience a wave of oocyte apoptosis as well as death of somatic ovarian tissue with loss of female specific gene expression that effectively converts their gonad into a testis. Ablation of the germ line via expression of toxins under the control of primordial germ cell-specific promoters or mutations in genes essential for primordial germ cell migration and viability, such as *dead end* and *nanos*, leads to loss of primordial germ cells by late somite stages and obligatory development of affected individuals into agametic males having only a microscopic remnant of the somatic portions of the testes. These findings have supported the notion that elements within germ cells, such as cytochrome P450 aromatase, are required for female sex determination. We have isolated a recessive zygotic mutation in zebrafish leading to loss of germline stem cells via apoptosis at approximately the time of sex determination. By 40 dpf mutants have lost almost all of their germ cells and the size of their gonads is greatly diminished. All mutants develop into agametic sterile male adults lacking visible testes. Mutant females are extremely rare yet are fertile consistent with the mutation specifically affecting germ cell viability during the time of sex determination. Efforts to determine the cell autonomy of the mutant gene as well as the dependence of germ cell loss on p53-mediated apoptosis are ongoing. Mutations in two factors required for piRNA biogenesis, Ziwi and Zili also lead to loss of the germline and development of agametic males. Accordingly, we are examining the status of transposable elements, the targets of piRNAs, in mutant gonads and are actively pursuing a positional cloning strategy to identify the mutant gene. We anticipate the discovery of a novel vertebrate factor required to maintain germ cell viability and hope to elucidate mechanisms of sex determination in the zebrafish, an increasingly utilized vertebrate model organism.

TRANSLATIONAL REPRESSION BY TESTIS-SPECIFIC ARGONAUTE PROTEIN PIWIL1

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Mammalian spermatogenesis is a highly specialized process of cellular differentiation. This process involves amplification of germline stem cells, differentiation into spermatocytes, meiotic divisions to produce haploid round spermatids, and transformation into spermatozoa. A large number of mRNAs undergoes post-transcriptional and translational regulation in the haploid phase of spermatogeneis, spermiogenesis. Small regulatory RNAs such as miRNA and siRNA associated with AGO subfamily proteins are known to repress gene expression at the post-transcriptional and translational levels. Another class of small RNA, called piRNA, is specifically expressed in germ line cells and is included in PIWI subfamily proteins. Mouse PIWI proteins, PIWIL2, PIWIL4, and PIWIL1, have been demonstrated to be essential for spermatogenesis. PIWIL2 and PIWIL4 function in transposon silencing by epigenetic mechanism during pre-meiotic and meiotic stages. However, the function of PIWIL1 is largely unknown. In this study, we show that PIWIL1 is associated with various polysomal mRNAs through a direct interaction with cytoplasmic poly(A)-binding protein PABPC1. When PIWIL1 was artificially tethered to the 3'-untranslated region of a luciferase reporter mRNA in cultured cells, translation was inhibited without reducing the mRNA level. Moreover, the N-terminal and PIWI domains of PIWIL1 were capable of directly binding PABPC1 and inducing translational repression. Although TNRC6 is required for gene silencing mediated by miRNA in AGO proteins, no interaction was observed between PIWIL1 and TNRC6. These findings suggest that PIWIL1 may function as a repressor at the post-initiation steps of translation in a TNRC6-independent manner.

The Histone Demethylase LSD1/KDM1 Regulates Stem Cell Fate by Reprogramming Epigenetic Memory

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Extensive epigenetic reprogramming occurs during cloning and in the induction of pluripotent stem cells (IPS). Similarly, during normal germline transmission, the epigenetic state of the genome is known to undergo extensive reprogramming both in the gametes and in the early embryo. This reprogramming may be essential to re-establish a developmental ground state between generations, but the mechanism of these epigenetic reprogramming events remains poorly understood. We show that mutants in *spr-5*, the C. elegans ortholog of the histone demethylase LSD1/KDM1 exhibit progressive sterility over many generations due to the transgenerational accumulation of the histone modification dimethylation of histone H3 on lysine 4 (H3K4me2). This suggests that H3K4me2 can serve as a stable epigenetic memory and that erasure of H3K4me2 by KDM1 in the germline prevents the inappropriate transmission of this epigenetic memory from one generation to the next. To pursue this role further, we generated mice with germline mutations in KDM1. Progeny from mice that lack maternal KDM1 exhibit significant embryonic and perinatal lethality. These progeny are genotypically normal, suggesting that the defects are due to the stable transmission of inappropriate histone methylation in the embryo. Furthermore, deletion of KDM1 in the mouse testis results in sterility, with loss of the testis stem cell population. These data suggest that KDM1 may be required both to maintain stem cell populations, as well as to restore totipotency after fertilization. In addition, KDM1 demethylation may be required for the somatic reprogramming that is induced during the generation of IPS cells.

ELUCIDATING GENETIC MECHANISMS BY WHICH SOMATIC CAMP SIGNALING REGULATES MSP-DEPENDENT OOCYTE GROWTH AND MEIOTIC MATURATION IN *C. ELEGANS*

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Oocyte meiotic maturation is a conserved developmental transition, defects in which are the major cause of human birth defects and infertility. *C. elegans* provides a genetic model for studying the control of oocyte meiotic maturation by hormonal signaling and soma-germline interactions. The meiotic maturation process in *C. elegans* and mammals share a number of similarities. Major sperm protein (MSP) and luteinizing hormone, though unrelated in sequence, both trigger meiotic resumption using somatic $G\alpha_s$-adenylate cyclase pathways and soma-germline gap-junctional communication. Shared responses include cortical cytoskeletal rearrangement, nuclear envelope breakdown, meiotic spindle assembly, and changes important for the oocyte-to-embryo transition. This poster describes progress in elucidating the genetic mechanisms by which the somatic gonad receives the MSP signal and transduces the oocyte response (the poster by C. Spike analyzes translational control mechanisms in meiotic maturation).

$G\alpha_s$-adenylate cyclase signaling in the follicle-like gonadal sheath cells is required for all described MSP responses in the germline. Because the gonadal sheath cells form gap junctions with oocytes, we considered whether cAMP generated in sheath cells might trigger cAMP-dependent protein kinase A (PKA) activation in the oocyte. We used genetic mosaic analysis to test this possibility. We found that *kin-1*, which encodes the PKA catalytic subunit, is required for meiotic maturation in the sheath cells, but not oocytes. Studies are in progress to test whether MSP increases cAMP levels in sheath cells using Epac2-camps300 cAMP sensors. To identify candidate sheath cell MSP receptors, we conducted a screen for GPCRs detectable in cDNA prepared from dissected gonads. This screen identified ~200 of the ~2000 *C. elegans* GPCRs. We next used recombineering to generate translational GFP fusions, which led to the identification of 22 sheath-cell expressed GPCRs. Mutant analyses and MSP binding assays of these 22 GPCRs are underway.

To identify effectors of MSP signaling, we conducted a genetic screen for mutations that can suppress the sterile phenotype caused by a null mutation in *acy-4*, which encodes the adenylate cyclase required in sheath cells for meiotic maturation. We recovered 63 suppressor of adenylate cyclase (*sacy*) mutations. We performed chromosome mapping of *sacy* mutations using snip-SNPs and are currently assigning complementation groups. We are using whole genome sequencing to identify *sacy* genes. We will report our progress in characterizing the *sacy* genes.

CHARACTERIZATION OF MOUSE MALE GERMLINE STEM CELL NICHE BY GENE EXPRESSION PROFILING USING LASER CAPTURE MICRODISSECTION

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Stem cells are tightly linked to their niche or microenvironment, which regulates their behaviors. The germline stem cell (GSC) niche in *Drosophila* and *Caenorhabditis elegans* containing easily identifiable GSCs has been extensively characterized on the molecular and genetic levels, revealing the importance of multiple signaling pathways and cellular processes. However, the mammalian GSC niche is less defined because unequivocal identification of GSCs has also not been achieved.
In the mouse testis, a subset of primitive spermatogonia termed "undifferentiated spermatogonia" or "Aundiff" includes stem cells that constitute an as-yet-unidentified subpopulation. The Aundiff populations are located on the basal membrane within the seminiferous tubules, and are biased toward vascular network in the interstitial tissue. Differentiating spermatogonia left these regions and dispersed throughout the basal compartment of the seminiferous tubules. According to these findings the mammalian GSC niche is likely to be located around the vascular-associated regions. However, no specialized niche substructure and their cellular components within the seminiferous tubules have not been identified.
To explore the detailed substructute and cellular components of the GSC niche, we initiated a comprehensive identification of the genes expressed around the vascular-associated regions within the seminiferous tubules. First, we isolated distinct cell-type populations between vasculature-associated regions and the other tubule bounding regions by Laser Capture Microdissection (LCM). Using these samples, we analyzed their gene expression differences by microarray. Until now, we verified 18 genes preferentially expressed around the vascular-associated regions by in situ hybridization. Within this subset, 2 and 16 genes were expressed in germ-line and somatic cells, respectively. We anticipate that characterization of spatio-temporal expression patterns corresponding to putative stem cells will lead to greater understanding of stem cell-niche interactions.

RSKS-1/S6K AND GERMLINE PROLIFERATION IN *C.ELEGANS*

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The development of multicellular organisms requires proper coordination of cell proliferation and specification. This coordination is particularly crucial during early germline development when the balance between these two processes establishes the stem/progenitor cell populations that ultimately produce gametes. However, the molecular mechanisms that control these processes are not well understood. Recently, our lab has shown that insulin/IGF-like receptor (IIR) signaling is required for robust larval germline proliferation. The TOR (Target of Rapamycin)-S6K(p70 ribosomal S6 kinase) pathway is responsive to insulin signaling and nutrition in mammals. However, in *C. elegans* the pathway is not well defined. We have found that a null mutation in *rsks-1(sv31)*, the worm homolog of S6K, reduces larval germline proliferation. The severity of this defect is similar to that seen with a mutation in the insulin/IGF-1 like receptor (IIR) gene *daf-2*. We further investigated the mechanism by which germ cell numbers were affected in *rsks-1(sv31)* null mutants. We found that while cell death is not elevated, *rsks-1(sv31)* mutants display significant reduction in both mitotic index and S-phase index, suggesting that *rsks-1* is required for robust larval mitosis. We also tested the hypothesis that the germline proliferation defect of *rsks-1(sv31)*, like the *daf-2(-)* defect, is dependent on *daf-16*/FOXO, and found that it is not. We are actively investigating both the molecular and cellular requirements for *rsks-1* activity for germline proliferation.

POSTOVULATORY EGG AGING LEADS TO DEADENYLATION OF A SPECIFIC SET OF MATERNAL MRNAS AND LOSS OF DEVELOPMENTAL POTENTIAL

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Vertebrate eggs remain fertilizable within a specific time window, but show a time dependent increase in developmental defects and embryonic death upon delayed fertilization in several vertebrates, including humans as well. Although this loss of developmental competence upon postovulatory egg aging was shown already more than 100 years ago the underlying molecular events are poorly understood. Currently this issue is most relevant as it is evident, that postovulatory aging may contribute to the adverse outcome of Artificial Reproduction Technology.
As most oogenesis control mechanisms in mammals have been first and most intensively studied in Xenopus and the molecular events in aging eggs may be similar in these species, we established the frog Xenopus tropicalis as an experimental model to study postovulatory egg aging.
Although 3-hour aged eggs remained high fertilizable and morphology did not change, delayed fertilization led to a dramatic increase in malformation and mortality. Since the genomes of the egg and zygote are not transcribed, the synthesis of proteins required for egg maturation and cleavage is controlled by changes in the poly(A) tail length of the maternal mRNAs. We assumed that aging of the egg might affect these mRNAs. Gene expression profiling revealed that 14% of the polyadenylated maternal transcripts, mainly housekeeping genes, were downregulated upon postovulatory aging. These transcripts were not degraded, implying deadenylation of a specific set of maternal mRNAs. The affected transcripts are characterized by a relatively short 3'UTR and a paucity of cytoplasmic polyadenylation elements (CPE) and polyadenylation signals (PAS). Furthermore, maternal mRNAs known to be deadenylated during egg maturation as well as after fertilization were preferentially deadenylated in aged eggs.
Taken together our analysis of aging eggs reveals that unfertilized eggs are in a dynamic state that was previously not realized. First, deadenylation of transcripts being typically deadenylated during egg maturation continues. This implies overripeness of the aged egg in the truest sense of the word. Second, transcripts being normally deadenylated after fertilization loose their poly(A) in the aged egg. This implies that the egg awaiting fertilization starts processes normally only observed after fertilization. We postulate that the imbalance of the polyadenylated maternal transcripts upon egg aging contributes to the loss of developmental potential.

INVESTIGATING THE FUNCTION OF A NOVEL GERM-CELL SPECIFIC TARGET FOR CYCLIC NUCLEOTIDES

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Cyclic nucleotides are important second messengers that control a variety of physiological functions. In particular, cAMP and cGMP play major roles in sperm physiology. Whereas mainly cGMP controls sperm chemotaxis in marine invertebrates, cAMP regulates the swimming behaviour of mammalian sperm. However, the role of cyclic nucleotides during sperm-cell development is not well established. Only a few eukaryotic target proteins for cyclic nucleotides have been identified so far: the protein kinases A and G (PKA, PKG), the family of cyclic nucleotide-gated channels (HCN and CNG channels) and the exchange protein directly activated by cAMP (EPAC). Surprisingly, none of them seems to play a major role during spermatogenesis although the enzymes that synthesize cAMP and cGMP, the adenylate and guanylate cyclases, can be found in sperm precursor cells.

We performed a database survey searching for unknown proteins containing a cyclic nucleotide binding-domain (CNBD) and identified in a testis-specific mouse EST-database a new protein that does not belong to any of the known families of target proteins for cyclic nucleotides. We called this protein SCNBP (soluble cyclic nucleotide binding-protein). It can be found in up to 40 different species ranging from ciona to human. In mouse, there is only one isoform, which is exclusively expressed in male germ cells in late spermatocytes and spermatids. In drosophila, there are three isoforms and at least two of them seem to be exclusively expressed in male germ cells. We are now trying to elucidate the role of the SCNBP during germ-cell development by generating *Scnbp* knock-out mice and flies. *Scnbp*-deficient male mice are subfertile, whereas their female counterparts display normal fertility. At the moment, we are analysing whether testis morphology, spermatogenesis and/or sperm function is altered.
Binding of cyclic nucleotides to the mSCNBP has been demonstrated by using fluorescence techniques. Further studies will show what the binding affinities for either cAMP or cGMP are.
In summary, we have identified a novel target for cyclic nucleotides, which seems to play an important role for male fertility.

PHOSPHOPROTEOME CHANGES DURING EGG ACTIVATION IN D. MELANOGASTER

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The transition from egg to embryo involves a number of highly regulated events including the resumption and completion of meiosis, poly-adenylation and translation of some maternal mRNAs stored in the egg, as well as degradation of other maternal mRNAs and proteins. Collectively, these events are referred to as “egg activation”.

Little to no transcription occurs at the time of egg activation, and most processes are directed by maternally deposited mRNAs and proteins, which are regulated at the translational and post-translational level. Since the activities of kinases MAPK and CamKII and of the phosphatase calcineurin are modulated during egg activation, we hypothesized that concerted and rapid changes in the phosphorylation state of maternally deposited proteins may drive the rapid shift in cellular state from mature oocyte to activated egg. Using both immobilized metal affinity chromatography (IMAC) and 2D-gel electrophoresis followed by mass spectrometry, we tested this hypothesis by examining changes in the phosphoproteome of Drosophila mature oocytes and activated eggs. We identified approximately 300 proteins whose phosphorylation state changes during egg activation. Proteins in a range of classes are phospho-modulated during this transition, and we find that there are more dephosphorylation events than new phosphorylations. We also find that egg-activation genes such as *sra* (calcipressin) and *cortex* (a subunit of the APC/C) regulate the phosphorylation status of several proteins at this time.

A RING FINGER IN THE C-TERMINAL CYTOPLASMIC DOMAIN OF *C. ELEGANS* SPE-42 IS REQUIRED FOR PROTEIN FUNCTION DURING FERTILIZATION.

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The *C. elegans spe-42* gene was identified in a previous genetic screen for mutants that render normally self-fertile hermaphrodites sterile due to sperm defects. Spermatogenesis in both male and hermaphrodite *spe-42* mutants proceeds normally, and spermatozoa from both sexes are indistinguishable from wild type sperm despite their inability to fertilize oocytes. These observations led to the hypothesis that *spe-42* is necessary for sperm-egg recognition, binding or fusion. SPE-42 is a predicted six-pass plasma membrane protein with both the N- and C-termini on the cytoplasmic face. A protein homology search revealed a putative RING finger in the C-terminal cytoplasmic domain; the aim of this study was to determine the extent to which this motif is necessary for protein function. Hermaphrodites homozygous for the *spe-42(tn1231)* mutation are completely sterile, but crossing a wild type *spe-42* transgene into this mutant genetic background restores fertility to greater than 60% of the wild type level. Transgenic constructs with alanine substitutions for each of the eight RING finger cysteine residues predicted to participate in Zn^{++} coordination were made and crossed into *spe-42(tn1231)* mutant worms. Each of the mutations had a severe effect on the ability of the transgene to restore fertility, as worms with all but one mutant transgene produced less than 10% of the progeny compared to worms with a wild type transgene. The exception was Cys 681, the first cysteine in the RING finger, which reduced fertility to 45% of the wild type level when mutated. The presence of another cysteine at position 678 suggests that this residue may be partially compensating for the loss of Cys 681 in the mutant transgene. These data along with published structures of other RING fingers were used to produce an *in silico* model of the SPE-42 RING finger. The model predicted that the side chains of several amino acids supported by the RING finger are available for protein-protein interactions, and these are currently being investigated.

MIRNA FUNCTION IN *DROSOPHILA* GERM CELL DEVELOPMENT

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Post-transcriptional regulation is of fundamental importance for germ line development in metazoan species. A key regulatory mechanism is mediated by a class of small RNAs referred to as microRNAs (miRNAs), which often bind to the 3□UTR of specific transcripts and promote degradation or translational repression of their targets. In *Drosophila*, primordial germ cells remain transcriptionally silent during their early development. Not surprisingly, therefore, gene regulation at the post-transcriptional level is particularly important during this stage of germ cell development. However, post-transcriptional regulation continues to be crucial during the later development of germ cells as well, particularly during gametogenesis.

Computational prediction and sequencing projects have identified over 150 putative miRNAs in *Drosophila*. The Cohen laboratory is currently completing a comprehensive knock-out (K.O.) mutation project with the goal of establishing deletion lines for most miRNAs. Because of the importance of post-transcriptional regulation during germ cell development, we have begun to screen this miRNA K.O. collection for germ line cell-related phenotypes, with a particular emphasis on oogenesis as well as maternal and zygotic effects on primordial germ cell formation, migration and survival. We will report and discuss our preliminary findings in these screens.

INCREASED CKIT ACTIVATION IN PGC DEVELOPMENT AND REPROGRAMMING

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The growth factor KitL and its receptor cKit are central to most aspects of primordial germ cell (PGC) development. However, it remains unclear how the coupling of this single ligand and receptor elicits multiple cellular responses in PGCs. A number of deletion and loss-of-function mutations in mice have revealed positive regulatory functions for cKit and KitL in proliferation, survival, migration of PGCs in addition to their role in the reprogramming of PGCs to pluripotent embryonic germ cells (EGCs). Another class of activating cKit mutations is frequently associated with various tissue-specific tumors in mice and humans. Our studies of a cKit activating mutant, $cKit^{\Delta V558}$, revealed an unexpected phenotype: whereas a single allele produces gastrointestinal stromal tumors (GIST) in 100% of mice but does not affect germ cell development, the PGCs in homozygous $cKit^{\Delta V558}$ mutant embryos are severely depleted by apoptosis from e11.5. This anomalous PGC behavior could arise from their different response to elevated cKit activity or because $cKit^{\Delta V558}$ does not function as an activating allele in the germline as previously demonstrated in intestinal cells and melanocytes. To distinguish between these possibilities, we generated compound mutants between $cKit^{\Delta V558}$ and $KitL^{Sl}$. Autoactivity of $cKit^{\Delta V558}$ in melanocytes was confirmed by a rescue of $KitL^{Sl/+}$ coat color defects in $KitL^{Sl/+}$; $cKit^{\Delta V558/+}$ adults. Similarly, in the germline, one $cKit^{\Delta V558}$ allele mitigated PGC loss in $KitL^{Sl/Sl}$ embryos, demonstrating mutant receptor function in the absence of ligand. On the other hand, exacerbation of the $cKit^{\Delta V558/\Delta V558}$ PGC phenotype by reduced *KitL* gene dosage indicates that *ΔV558* retains some ability to respond to ligand in spite of its autoactivity. These genetic experiments support the hypothesis that PGCs are uniquely hypersensitive to cKit activity and raise the possibility of a downstream molecular "circuit breaker". One such mechanism we have observed in wild type PGCs is the downregulation of surface cKIT expression following KITL treatment, and the perturbation of this negative feedback loop in $cKit^{\Delta V558/+}$ PGCs. In order to ask biochemical questions about the downstream signaling pathways altered in $cKit^{\Delta V558}$ genetic backgrounds, we have generated EGC lines from the mutant PGCs. Studying the reprogramming efficiency, ligand responsiveness, and differentiation of mutant EGCs to PGCs, we will begin to connect specific downstream cKit pathways to cellular outcomes. If identified, a germ cell-specific cKit signal "circuit breaker" would exemplify a novel molecular mechanism for the avoidance of germ cell tumor formation.

MULTI SEX COMBS (MXC) REGULATES HISTONE MRNA SYNTHESIS AND GERM CELL BEHAVIOR IN THE *DROSOPHILA* TESTIS

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Strong loss-of-function mutations in the *Drosophila multi sex combs (mxc)* gene result in larval lethality. However, flies carrying a viable, hypomorphic mutation in mxc (mxc^{G46}) exhibit defects in hematopoiesis, as well as sterility of both males and females. Gonads from hemizygous mxc^{G46} males or mxc^{G46}/mxc^{22a-6} females are smaller and have fewer germ cells than wild type gonads. In males, there is a significant réduction in the number of spermatogonial cysts that often contain less than 16 cells. Clonal analysis using the null mxc^{22a-6} allele further revealed that *mxc* is required cell autonomously for germline stem cells (GSC) maintenance. Rescue of mutations in *mxc* with a GFP-tagged *mxc* transgene showed that MXC localizes specifically to the Histone Locus Body (HLB) on chromosome 2, the site for both histone mRNA transcription and processing in *Drosophila*. Using immunofluorescence and biochemical approaches, we find that mutations in *mxc* disrupt proper HLB formation and impairs histone production. These results provide a mechanistic link between histone synthesis, cell cycle progression, and the maintenance of the germline in *Drosophila*.

GERM CELL APOPTOSIS AND THE FORMATION OF CYTOPLASMIC GRANULES UNDER STRESS CONDITIONS IN *C. ELEGANS*: IS THERE SOME CONNECTION?

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During oogenesis in *C. elegans* an unknown mechanism eliminates half of germ cells in a process known as physiological apoptosis. Conditions like heat shock, oxidative and osmotic stresses increase germ cell apoptosis independently of pro-apoptotic proteins EGL-1 and p53, and through the MAPKK´s pathway. Starvation also induces apoptosis but by an unknown mechanism. In a microarray analysis, we identified genes whose expression changed during starvation. By RNAi, we are testing these genes function in germ cell apoptosis. We found several RNA binding proteins that associate to cytoplasmic ribonucleoprotein complexes implicated in post-transcriptional regulation of mRNAs. Among proteins found are CGH-1, CAR-1 and GLA-3 whose absence has been previously reported to trigger physiological germ cell apoptosis. CEY-2 has a cold-shock/Y-box domain and associates with CGH-1. PGL-1 and GLH-4 are key P granule components important for germline function. MEX-5, NOS-2, SPN-4, and OMA-1 are important for embryogenesis and/or oogenesis while IFE-1 is one of the five isoforms of eIF4E. Silencing of *cey-2, mex-5* and *pgl-1* triggered physiological germ cell apoptosis. This suggests that these genes protect directly or indirectly germ cells from physiological apoptosis. However under stress conditions no increase in apoptosis is observed when silencing *car-1*, *cgh-1*, *cey-2*, *mex-5*, *pgl-1* and *gla-3* suggesting that these genes are involved for this response. We did not observed any significant effect on germ cell apoptosis when *nos-2*, *spn-4*, *ife-1*, *oma-1* and *glh-4* were silenced. During stress conditions, like heat shock or starvation, large granules are formed in the core of the C. elegans gonad. Using an antibody against CGH-1, we tested if silencing of these RNA binding proteins had an effect on granules formation in normal and stress conditions. RNAi in *cey-2*, *mex-5*, *car-1*, *pgl-1* and *ife-1* induced the formation of granules even in the absence of stress while had no effect under stress conditions. Interestingly, silencing of *gla-3* inhibited the formation of granules during starvation and heat shock. GLA-3 is part of the TTP family of proteins whose members are important to assemble stress granules in mammals suggesting that in C. elegans has a conserved role. We are currently studying if there is a link between stress conditions, RNA granule formation and apoptosis.

VASA ACTIVATES *MEI-P26* TRANSLATION THROUGH A DIRECT INTERACTION WITH A (U)-RICH MOTIF IN ITS 3' UTR

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Vasa (Vas) is a DEAD-box RNA binding protein required in *Drosophila* at several steps of oogenesis and for primordial germ cell (PGC) specification. Vas associates with eukaryotic initiation factor 5B (eIF5B) and this interaction has been implicated in translational activation of *gurken* mRNA in the oocyte. Vas is expressed in all ovarian germ line cells, and aspects of the *vas*-null phenotype suggest a function in regulating the balance between germ line stem cells (GSCs) and their fate-restricted descendants. We used a biochemical approach to recover Vas-associated mRNAs, and obtained *mei-P26*, whose product represses microRNA activity and promotes GSC differentiation. We found that *vas* and *mei-P26* mutants interact, and that *mei-P26* translation is substantially reduced in *vas* mutant cells. In vitro, Vas protein bound specifically to a (U)-rich motif in the *mei-P26* 3'-untranslated region (3' UTR), and Vas-dependent regulation of *GFP-mei-P26* transgenes in vivo was dependent on the same (U)-rich 3' UTR domain. The ability of Vas to activate *mei-P26* expression in vivo was abrogated by a mutation that greatly reduces its interaction with eIF5B. Taken together, our data support the conclusion that Vas promotes germ cell differentiation by directly activating *mei-P26* translation in early-stage committed cells. We have produced further in vitro evidence that Vas interacts with other mRNAs that contain (U)-rich motifs and that localize to the pole plasm. We are working to develop ovarian extract assays that recapitulate Vas-mediated translational regulation.

LIS-1 AND *ASUNDER* COOPERATE TO REGULATE DYNEIN LOCALIZATION DURING *DROSOPHILA* SPERMATOGENESIS

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Mutations in human *LIS1* cause a brain malformation disorder known as Lissencephaly ("smooth brain"). Early genetic studies involving mutation of *LIS1* homologs in Aspergillus and yeast revealed that *LIS1* is essential for nuclear migration mediated by dynein, a minus-end-directed microtubule motor complex. *LIS1* is also required for the cell cycle functions of dynein, including centrosome movements, nuclear envelope breakdown, spindle assembly, and chromosome segregation. LIS1 protein has been shown to directly bind dynein as well as dynactin (an activator of dynein), although the precise role of LIS1 in regulating dynein function remains unclear.

Previous work from our lab and others has suggested that dynein plays a critical role in nucleus-centrosome coupling and cell cycle progression in *Drosophila* spermatogenesis. To assess the role of the *Drosophila* homolog of *LIS1* (*Lis-1*) in these processes, we have characterized the male-sterile phenotype of flies homozygous for a hypomorphic allele of *Lis-1* (*Lis-1*^{k11702}). *Lis-1* males have germline defects previously associated with decreased dynein function (e.g. loss of nucleus-basal body coupling in spermatids) and some new phenotypes. Centrosomes of *Lis-1* spermatocytes fail to break their association with the cell cortex to migrate to the nuclear surface during late G2; aberrant meiotic spindles with cortical centrosomes form as a result. The Nebenkerne (mitochondrial aggregates) of *Lis-1* spermatids exhibit abnormal morphology and loss of attachments to the nucleus and basal body. These data suggest additional roles for dynein in regulating centrosomes and mitochondria during spermatogenesis.

LIS-1 co-localizes with dynein-dynactin during spermatogenesis, and dynein-dynactin fails to localize to the nuclear surface of *Lis-1* spermatocytes and spermatids. We previously identified *asunder* (*asun*) as a critical regulator of dynein-dynactin localization during *Drosophila* spermatogenesis. *Lis-1* is a strong dominant enhancer of *asun* in the male germ line, suggesting that these genes cooperate in the regulation of dynein during spermatogenesis. Our preliminary data indicate that *asun* germline cells show reduced perinuclear localization of LIS-1, whereas ASUN localization is normal in *Lis-1* testes. We present a model in which ASUN and LIS-1 act sequentially to recruit dynein-dynactin to the nuclear surface, an essential step to ensure proper positioning of centrosomes at meiotic entry and fidelity of meiotic divisions.

DIFFERENTIATION AND GENE EXPRESSION IN FEMALE GERMLINE IN THE SOUTH AMERICAN PLAINS VIZCACHA, *LAGOSTOMUS MAXIMUS.*

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In mammals, Primordial Germ Cells (PGCs) originates from the proximal epiblast, proliferate and migrate reaching the gonadal ridges. This process is regulated by a sequential gene expression, most notably *Blimp1, Oct4, Fragilis, Nanos, Stella* and *Vasa*. All of them play an essential role in maintaining pluripotency and in germ cell destiny and maturation. We analyzed the expression of the germline markers *Stella*, a protein with a SAP-like domain and a splicing factor motif-like structure, *Vasa* a protein encoding a DEAD (Asp-Glu-Ala-Asp)-family protein of putative RNA helicases and the transcription factor *Oct4* during germ cell differentiation, migration, colonization and proliferation in *L. maximus*, a caviomorph rodent displaying the highest ovulation rate described for a mammal (up to 800 oocytes in each reproductive cycle). Massive polyovulation arises from the overexpression of the apoptosis-inhibiting BCL2 gene leading to a suppression of apoptotic pathways responsible for follicular atresia in mammals and germ cell attrition in the developing ovary. 34 pregnant adult females of *L. maximus* were captured from a natural population at the Estación de Cría de Animales Silvestres (ECAS), Buenos Aires province, Argentina, during the main breeding season. We studied 15 embryos before/during gonad formation, 12 fetal ovaries and 5 pre-puber ovaries by immunohistochemistry, western blot and whole-embryo immunohistochemistry. *OCT4* expression was confined to the epiblast compartment in pre-somite embryos. At the beginning of gastrulation, *OCT4* was expressed in PGCs (n <90) located at the base of the allantois. *STELLA* and *VASA* were not detected in those embryo stages. Germ cells then migrated along the dorsal mesentery and proliferated (n >180 PGCs) by mitosis with a nuclear expression of *STELLA* and *OCT4*, and cytoplasmic expression of *VASA*. Once in the ovary, *OCT4* was expressed in oogonia (n>600 PGCs), but it was down-regulated in meiotic oocytes. Then its expression became evident in primordial, primary and secondary follicles. *STELLA* was expressed in the nucleus of oogonia and in oocytes forming primordial follicles. *VASA* was strongly expressed in the cytoplasmic of germ cells throughout folliculogenesis. Both in fetal and post-natal ovary, two groups of cells were evident: mitotically active germ cells showing an immature phenotype and follicle enclosed germ cells. A different pattern of the germ cell markers expression was correlated to migration, proliferation, profase I, and folliculogenesis. Compared to other mammals, those gene expression patterns were species-specific, and seem to indicate a role in primordial germ cell differentiation, destiny and maturation and survival.

SERPINE2, A SERINE PROTEASE INHIBITOR EXTENSIVELY EXPRESSED IN ADULT MALE MOUSE REPRODUCTIVE TISSUES, MAY SERVE AS A MURINE SPERM DECAPACITATION FACTOR

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SERPINE2, one of the potent serine protease inhibitors that modulates the activity of the plasminogen activator and thrombin, is implicated in many biological processes. In the present study, we purified SERPINE2 from mouse seminal vesicle secretion (SVS) using liquid chromatography and identified it by liquid chromatography/tandem mass spectrometry, and it showed potent inhibitory activity against the urokinase-type plasminogen activator. SERPINE2 was predominantly expressed in seminal vesicles among murine male reproductive tissues. It was immunolocalized to the SVS and mucosal epithelium of the seminal vesicle, epididymis, coagulating gland, and vas deferens. In the testes, SERPINE2 was immunostained in spermatogonia, spermatocytes, spermatids, Leydig's cells, and spermatozoa. SERPINE2 was also detected on the acrosomal cap of testicular and epididymal sperm and was suggested to be an intrinsic sperm surface protein. The purified SERPINE2 protein could bind to epididymal sperm. A prominent amount of SERPINE2 was detected on ejaculated and oviductal spermatozoa. Nevertheless, SERPINE2 was only detected on uncapacitated sperm, not on capacitated sperm, indicating that SERPINE2 is lost before initiation of the capacitation process. Moreover, SERPINE2 could inhibit in vitro bovine serum albumin-induced sperm capacitation and prevent sperm binding to the egg, thus blocking fertilization. It acts through preventing cholesterol efflux, one of the initiation events of capacitation, from the sperm. These findings suggest that the SERPINE2 protein may play a role as a sperm decapacitation factor.

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GENOME STABILITY AND DNA DAMAGE SIGNALING IN 129-*DND1*$^{TER/TER}$ MURINE TESTICULAR TERATOMAS

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Testicular germ cell tumors (TGCTs) are the most common cancer diagnosed in young men and constitute the majority of testicular malignancies. TGCTs are among the most curable cancers following treatment with DNA-damaging chemotherapies, with a strikingly high cure rate of over 90% for newly-diagnosed cancers. In most pre-invasive solid tumors, DNA damage checkpoint factors are constitutively activated in response to the DNA damage associated with oncogenic stress and increased proliferation. TGCTs in humans, on the other hand, appear to completely lack activation of these factors. We hypothesize that the unique sensitivity of TGCTs to chemotherapy is due to differences in the DNA damage response in the germ cells from which TGCTs originate. In order to test this hypothesis in the mouse, we investigated genome stability and the DNA damage response in 129-*Dnd1*$^{Ter/Ter}$ mice, which have a high incidence of testicular teratomas. Cells cultured from *Dnd1*$^{Ter/Ter}$ teratomas were largely diploid with a moderate range of variation and a subset of nuclei with hypotetraploid chromosome numbers. Higher resolution chromosome analysis via array comparative genome hybridization (aCGH) revealed that despite the nearly diploid chromosome number, *Dnd1*$^{Ter/Ter}$ tumors harbored significant genome alterations including recurrent amplifications. Assessment of DNA damage checkpoint activation in *Dnd1*$^{Ter/Ter}$ tumors and cultured *Dnd1*$^{Ter/Ter}$ cells is in progress. Together, this work will help to elucidate the molecular mechanisms responsible for the exquisite curability of TGCTs and will further our understanding of how the germline responds to DNA damage.

REGULATION OF MITOCHONDRIAL DNA ACCUMULATION DURING OOGENESIS

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Oocytes contain a large stock of mitochondria. These are essential because, following fertilization, the embryo does not resume mitochondrial DNA (mtDNA) replication until near the time of implantation; therefore, a large stock is essential to ensure that each blastomere inherits a sufficient number of mitochondria. However, the timing and control of mtDNA accumulation in oocytes are poorly understood. We have developed a PCR-based assay that enables the mtDNA content of individual mouse oocytes to be measured. We report that the quantity of mtDNA increases progressively during oocyte growth, reaching ~175,000 copies per cell. When oocytes reach full-size, however, mtDNA ceases to accumulate. Thus, accumulation of mtDNA is closely correlated with oocyte growth. To investigate the mechanism of accumulation, we analyzed mtDNA content in mouse oocytes grown in vitro. As in vivo, we found that mtDNA content increased during oocyte growth in vitro. Unexpectedly, although oocytes did not grow to the same size in vitro as in vivo, the mtDNA accumulated to the same extent under both conditions. This suggests that mtDNA accumulation is mechanistically uncoupled from oocyte growth. To test this, we incubated growing oocytes in the presence of LY294002, an inhibitor of phosphoinosotide-3 kinase, or in the absence of the surrounding granulosa cells. As expected, oocyte growth was inhibited under both conditions. In contrast, mtDNA accumulated in the oocytes despite the growth inhibition. These results indicate that the accumulation of mtDNA is independent of oocyte growth. We then examined the expression of nuclear-encoded genes required for mtDNA replication. The amount of Tfam, Polg A, and Polg B increased co-ordinately with oocyte growth. Unexpectedly, however, their quantity subsequently declined in fully grown oocytes. We propose that mtDNA ceases to accumulate in fully grown oocytes owing to the loss of the mRNAs encoding these essential components of the replication machinery. Thus, these mRNAs may be limiting factors that determine the rate and extent of mtDNA accumulation during oogenesis.

REGULATION OF CHROMATIN STRUCTURE IN *C. ELEGANS* MEIOTIC GERM CELLS BY A SMALL RNA PATHWAY

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Small RNA-mediated pathways are essential for germline development in *C. elegans* where they have been implicated in many aspects of gene regulation. We have identified a role for small RNAs in regulating accumulation of the chromatin mark, H3K9me2 (histone H3 lysine 9 dimethylation), during meiosis. In *C. elegans*, H3K9me2 levels are low in mitotic nuclei and remain low on chromosomes that undergo homologous pairing and synapsis during prophase of meiosis I. In contrast, chromosomes that do not undergo homologous pairing and synapsis, such as the male X, accumulate a high level of H3K9me2 during pachytene stage. Although its specific function is unclear, studies in many species suggest that the H3K9me2 mark often correlates with reduced transcription. In *C. elegans* germ cells, elevated H3K9me2 is considered an indicator of meiotic silencing of unpaired chromatin, a general phenomenon described in many animal species. Chromosomes other than the male X are also enriched for H3K9me2 during pachytene if their homologous pairing or synapsis is disrupted by mutation or chromosomal duplication. We previously found that the meiotic H3K9me2 accumulation pattern depends on components of the germline small RNA machinery, including EGO-1 (RNA-dependent RNA pol), EKL-1 (Tudor domain protein), DRH-3 (helicase), and CSR-1 (Argonaute). H3K9me2 does not accumulate to the normal level on unpaired chromosomes during pachytene stage in *ego-1*, *ekl-1*, *drh-3*, or *csr-1* XO or XX mutant germ lines, and ectopic H3K9me2 is observed on paired chromosomes in *ekl-1*, *drh-3*, and *csr-1* mutants. These four factors are known to constitute a distinct small RNA pathway, which we hypothesize acts at the chromatin level to regulate activity of the histone methyltransferase responsible for germline H3K9me2, MET-2. We are taking genetic and biochemical approaches to identify additional factors acting in conjunction with this small RNA pathway to regulate targeting and accumulation of H3K9me2 marks during meiosis. Among factors that associate physically with EKL-1 are chromatin proteins as well as components of the small RNA machinery. We have also identified a role for histone deacetylase (HDAC) activity in the meiotic silencing process. Mutations in (some) HDAC complex components disrupt meiotic H3K9me2 accumulation in certain contexts. We are taking a genetic approach to determine the specific situations in which HDAC activity promotes meiotic silencing and to identify other HDAC components that are required.

MECHANISMS ESTABLISHING OOCYTE ASYMMETRIES AND THE ANIMAL-VEGETAL AXIS IN ZEBRAFISH.

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The vertebrate animal-vegetal embryonic axis is established during oogenesis. Establishing oocyte polarity is crucial for determining the prospective embryonic axes and setting aside the germ cell determinants in non-mammalian vertebrates. The Balbiani body is an evolutionarily conserved oocyte asymmetry present in early oocytes of all animals examined, including humans. Although identified more than 150 years ago, genes acting in Balbiani body assembly are largely unknown in vertebrates. Bucky ball was identified as an essential regulator of oocyte polarity through maternal-effect genetic screens. In zebrafish *bucky ball* mutants, mRNAs including germ plasm components are not localized properly in oocytes, and the Balbiani body does not form.

Polarized localization of Buc protein in pre-Balbiani body stage oocytes indicates that oocyte polarity may be established very early in oogenesis and that Buc acts prior to or initiates Balbiani body development. To investigate how Bucky ball regulates Balbiani body formation and mRNA and other asymmetries in oocytes we are identifying components of the Bucky ball pathway. Recently we have identified Bucky ball-binding proteins which link Bucky ball to RNA binding. We are determining which regions of the Buc protein are important for engaging binding partners to potentially reveal regions of functional significance, which are not known and cannot be predicted for Bucky ball. We are using *bucky ball* mutants to investigate the function of Buc interacting proteins and to gain insight into the mechanisms underlying Balbiani body formation and oocyte polarity, including asymmetric localization of mRNAs.

THE TESTIS REGULATOR DMRT1 MAINTAINS SEXUAL IDENTITY AND CELL FATE IN MALE NEONATAL GERM CELLS

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DMRT1 is a Doublesex-related transcription factor that controls testis differentiation in vertebrates. DMRT1 is expressed in germ cells and Sertoli cells and our previous work has shown that it has multiple requirements in both cell types. To further explore the role of DMRT1 in germ cells we have used *Ngn3-cre* to delete *Dmrt1* in neonatal germ cells soon after birth. At this stage *Ngn3-cre* is active in a fraction of precursors of the germ line stem cell population that contributes to adult spermatogenesis. *Ngn3-cre* is not expressed in somatic cells of the testis.

The mutant cells failed to express any of several germ cell-specific proteins, suggesting that they had lost germ cell identify. However, the mutant cells did not express the Sertoli cell proteins GATA1 and SOX9. They did express GATA4, which normally is expressed in somatic cells of the testis and ovary. Remarkably, the mutant cells robustly expressed the granulosa cell proteins FOXL2 and LRH1 and had nuclear morphology similar to that of granulosa cells, strongly suggesting that they were transformed from male germ cells into female somatic cells. These mutant granulosa-like cells may function as female steroidogenic cells: they expressed the steroidogenic transcription factors AR, DAX1, and SF1, and the mutant testes had elevated expression of aromatase (*Cyp19a1*, which synthesizes estradiol) and elevated expression of estrogen-induced mRNAs. Chromatin immunoprecipitation demonstrated that DMRT1 binds DNA regions near the promoters of *Ar* and *Nr5a1/Sf1*, suggesting that DMRT1 directly represses these steroidogenic factors in male germ cells. In support of this view, known SF1 target mRNAs were elevated in conditionally targeted mutant testes at P9.

These results demonstrate that DMRT1 is required in neonatal germ cells to maintain both their male identity and their commitment to germ cell development, and reveal remarkable developmental plasticity in these cells. Clearly commitment to the male fate has to be actively maintained in male germ cells even after birth. Our data also require a re-evaluation of the origin of granulosa cell tumors of the testis, which previously have been assumed to be of somatic origin.

PROGRAMMING AND REPROGRAMMING GERM CELL FATES

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The long-standing interest of my laboratory is the molecular regulation of germ cell fate decisions. How are germline stem cells (GSCs) controlled? What regulates germ cells to enter the meiotic cell cycle? And perhaps most mysterious at the current time, what regulates their decision to differentiate as sperm or oocyte? Our approach has been to investigate these decisions in the nematode *C. elegans*, where each fate choice has been tractable to genetic, molecular and most recently, genomic analyses. Importantly, *C. elegans* adult germ cells are continuously controlled to maintain GSCs, enter the meiotic cell cycle and differentiate as sperm or oocyte. My talk will report recent advances in our understanding of the molecular controls of germ cell fates and their reprogramming.

REGULATION OF PROLIFERATION AND DIFFERENTIATION IN THE GERM LINE STEM CELL LINEAGE

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Germ line stem cells maintain production of male and in some organisms female gametes throughout reproductive life in many organisms. In adult stem cell lineages, progenitor cells commonly undergo several mitotic transit amplifying (TA) divisions before terminal differentiation, allowing production of many differentiated progeny per stem cell division. The mechanisms that stop TA divisions and mediate the switch to differentiation may provide important protection from cancer by minimizing accumulation of oncogenic mutations. The Drosophila germ line provides a powerful system to study mechanisms that regulate self-renewal, proliferation, and differentiation in adult stem cell lineages. The bag of marbles (bam) protein and its partner benign gonial cell neoplasm (bgcn) are required in male germ cells for spermatogonia to switch from TA cell proliferation to the spermatocyte program, meiosis and terminal differentiation. Bam protein accumulates in 4-cell spermatogonial cysts, reaches a peak in 8-cell cysts and is abruptly degraded in early 16-cell spermatocyte cysts, immediately after premeiotic S- phase. The number of TA divisions appears to be set by the accumulation of Bam protein to a critical threshold. The switch from TA cell proliferation to differentiation in the Drosophila male germline adult stem cell lineage is mediated by a translational control cascade. The Drosophila TRIM-NHL tumor suppressor homolog and microRNA regulator Mei-P26 facilitates accumulation of the differentiation regulator Bam in TA cells. In turn, Bam and its binding partner Bgcn repress translation of mei-P26 in late TA cells via the mei-P26 3'UTR. TRIM-NHL homologs in D. melanogaster, C. elegans, and M. musculus are required for the switch from proliferation to differentiation in stem cell lineages, suggesting a common developmentally-programmed tumor suppressor mechanism. The switch from meiosis to mitosis sets up a further translational control system that shapes the meiotic cell cycle, delaying production of cell cycle regulators cdc25 and cyclin B until genes required for spematid differentiation are expressed and allowing the extended G2 phase of meiotic prophase.

GENOME-WIDE ANALYSES REVEAL A ROLE FOR PEPTIDE HORMONES IN PLANARIAN GERMLINE DEVELOPMENT

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Bioactive peptides (i.e. neuropeptides or peptide hormones) represent the largest class of cell-cell signaling molecules in metazoans, and are potent regulators of neural and physiological function. In vertebrates, peptide hormones play an integral role in endocrine signaling between the brain and the gonads that controls reproductive development, yet few of these molecules have been shown to influence reproductive development in invertebrates. Here, we define a role for peptide hormones in controlling reproductive physiology and germ cell development in the planarian *Schmidtea mediterranea*. Based on our observation that defective neuropeptide processing results in defects in germ cell differentiation, we employed peptidomic and functional genomic approaches to characterize the planarian peptide hormone complement, identifying 51 prohormone genes and validating 142 peptides biochemically. Comprehensive *in situ* hybridization analyses of prohormone gene expression revealed the unanticipated complexity of the flatworm nervous system and identified a prohormone specifically expressed in the nervous system of sexually reproducing planarians. We show that this member of the neuropeptide Y superfamily is required for the maintenance of mature reproductive organs and differentiated germ cells in the testes. These studies describe the peptide hormone complement of a flatworm on a genome-wide scale and reveal a previously uncharacterized role for peptide hormones in coordinating flatworm germ cell dynamics.

MUSASHI FAMILY OF RNA BINDING PROTEINS: CELL CYCLE REGULATORS IN SPERMATOGENESIS.

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Mammalian meiosis is a tightly regulated process involving specialized cell cycle progression and morphogenetic changes. We have demonstrated that the Musashi family of RNA binding proteins is implicated in the regulation of spermatogonial stem self renewal and germ cell differentiation. Here we describe the novel mechanism by which the Musashi family proteins, Msi1 and Msi2, act to control exit from spermatogonial mitotic amplification and normal entry into meiosis. Gene and protein analysis indicated overlapping Msi1 and Msi2 profiles in enriched populations of isolated germ cells and reciprocal subcellular expression patterns in spermatogonia and pachytene spermatocytes/ round spermatids in testes sections. Recombinant Msi1 protein-RNA pulldown and microarray analysis coupled with in vitro shRNA knockdown studies in spermatogonial culture and subsequent immunoprecipitation and qPCR established that Msi1 targeted Msi2 mRNA for post transcriptional translational repression. Immunoprecipitation of Msi2 target mRNA and subsequent qPCR together with in vitro shRNA knockdown studies in round spermatid culture identified a cell cycle inhibitor protein CDKN1C ($p57^{kip2}$) as the principal target of Msi2 translational inhibition. Immunolocalisation of CDKN1C protein indicated that expression of this cell cycle regulator coincided with the nuclear import of Msi1 and the appearance of cytoplasmic Msi2 expression in early pachytene spermatocytes. Using a transgenic Msi1 overexpression mouse model in conjunction with quantitative gene and protein expression, we confirmed Msi1 targeting of Msi2 and subsequent Msi2 targeting of CDKN1C for translational repression in vivo. Ectopic overexpression of Msi1 in germ cells induces substantial Msi2 downregulation and aberrant CDKN1C expression, resulting in abnormal spermatogenic differentiation, germ cell apoptosis/arrest and sterility. In conclusion, our results indicate a sophisticated molecular switch encompassing cell cycle protein regulation by Musashi family proteins, is required for normal exit from mitotic division, entry into meiosis and post meiotic germ cell differentiation.

REGULATION OF CELL CYCLE ARREST BY DND1 IS MODULATED BY GENETIC BACKGROUND

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The classic mouse mutation, *Ter*, named for the high incidence of testicular teratomas it causes on the 129/SvJ strain, is a nonsense mutation in the RNA binding protein, *Dead end 1* (*Dnd1*^{Ter}). *Dnd1*$^{Ter/Ter}$ causes a severe loss of germ cells on all strain backgrounds early in embryogenesis. Although a similar small number of germ cells escape this fate and reach the gonad in all strains tested, spontaneous testicular teratomas arise only on the 129/SvJ strain in *Dnd1*$^{Ter/Ter}$ mutants. Introduction of a null allele of the pro-apoptotic gene, *Bax* (*Dnd1*$^{Ter/Ter}$*;Bax*$^{-/-}$), rescues much of the early germ cell loss, and leads to an increase in the number of gonadal germ cells. This results in testicular teratomas on most genetic backgrounds, however, no tumors arise on C57BL/6J. On susceptible strains, many mutant germ cells fail to enter mitotic arrest in G0, do not downregulate the pluripotency markers, NANOG, SOX2, and OCT4, and initiate teratoma formation by E15.5. We show that mouse DND1 directly binds a group of transcripts that encode negative regulators of the cell cycle, including *p27*^{Kip1} and *p21*^{Cip1}, neither of which is translated in *Dnd1*$^{Ter/Ter}$ germ cells. This strongly suggests that DND1 regulates mitotic arrest in male germ cells through translational regulation of cell cycle genes. Like all other strains tested, C57BL/6J *Dnd1*$^{Ter/Ter}$*;Bax*$^{-/-}$ germ cells fail to arrest in G0, yet they block entry into M-phase, unlike mutant germ cells on 129/SvJ and mixed backgrounds. Coincident with arrest prior to M-phase, C57BL/6J mutant germ cells successfully downregulate pluripotency markers. This work suggests that reprogramming of pluripotency in germ cells and prevention of tumor formation requires cell cycle arrest. Strain-specific differences in the regulation of cell cycle may account for the differences in sensitivity to testicular tumors characteristic of the human population.

CYCLIN E / CDK-2 REGULATES PROLIFERATIVE CELL FATE AND CELL CYCLE PROGRESSION IN THE *C. ELEGANS* GERMLINE

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Two important aspects of stem cells are the regulation of cell fate and the regulation of proliferation rate. In contexts where cell proliferation is a defining aspect of a particular cell fate, genes that regulate cell proliferation may also regulate cell fate. To investigate this possibility, we analyzed the role of cell cycle factors in the C. elegans germline, where germ cells execute either a proliferative or meiotic development cell fate. We found that cyclin E and cdk-2 promote the proliferative fate, in addition to regulating progression through the mitotic cell cycle. Cyclin E is expressed in proliferative cells of the germline but targeted for degradation upon entry into meiosis. As evidence that cyclin E and cdk-2 play an important role in promoting the proliferative fate, we find that a reduction in cyclin E or CDK-2 by RNAi causes proliferative cells in a sensitized genetic background to enter meiosis. Furthermore, cyclin E acts downstream of known signaling pathways that regulate this cell fate decision in the C. elegans germline.

Additional characterization of cell cycle progression among proliferative cells in the germline provides details that may explain how cyclin E/cdk-2 plays a central role in both processes. By analyzing cell cycle kinetics, we identified three important characteristics of proliferative cells: 1) continuous cell cycle progression, 2) relatively rapid cell cycle progression and 3) a unique cell cycle structure that lacks a significant G1 phase. Consistent with other contexts where G1 appears absent, we find that cyclin E levels are not periodic during cell cycle progression but remain high throughout. We propose that constitutively high cyclin E allows these cells to bypass G1 regulation, thereby playing a role in their rapid, continuous cell cycle progression. Since cyclin E degradation is not coupled to a specific cell cycle stage, the presence or absence of cyclin E can serve as a regulatory cue for the proliferation versus meiotic entry cell fate decision.

OVEREXPRESSION OF CONSERVED RNA-BINDING PROTEINS PROMOTES MEIOTIC PROGRESSION IN GERM CELLS DIFFERENTIATED FROM PLURIPOTENT STEM CELLS

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Ten to fifteen percent of couples are infertile because of problems linked to the production of few or no oocytes or sperm. Yet our understanding of human germ cell development is poor, at least in part due to inaccessibility of early developmental stages to molecular genetic studies. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) may provide a human genetic system to study germ line development and ultimately assist with infertility. Both hESCs and iPSCs can form cells of all three embryonic germ layers, endoderm, mesoderm and ectoderm, and also germ cells in vitro. In this study we tested whether overexpression of the germ cell specific factors DAZ, DAZL, BOULE and VASA altered differentiation of pluripotent cells. We observed that both hESCs and iPSCs spontaneously differentiated to primordial germ cells (PGCs); moreover, they formed meiotic and post-meiotic cells only when subjected to our overexpression system. These results suggest that not only the DAZ, DAZL and BOULE genes play a critical role in meiotic regulation from hESCs as previously described, but also that VASA overexpression can also promote formation of haploid cells in both hESCs and iPSCs. These data may provide a useful model for basic human molecular genetic studies in germ line formation and inform future clinical studies of potential novel therapeutic applications.

THE MAMMALIAN DOUBLESEX HOMOLOG DMRT1 IS A TRANSCRIPTIONAL GATEKEEPER THAT CONTROLS THE MITOSIS VERSUS MEIOSIS DECISION IN MALE GERM CELLS

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In mammals, initiation of meiosis requires retinoic acid (RA), which activates meiotic inducers including Stra8. However, control of the choice between mitosis and meiosis in male germ cells (spermatogonia) remains poorly understood. We have used conditional gene targeting and chromatin immunoprecipitation (ChIP) to investigate the functions of the Doublesex-related transcription factor DMRT1 in mammalian testis development.
Our previous work showed that DMRT1 plays a number of distinct roles in the development and function of both germ cells and Sertoli cells and that the mutant phenotype is highly dependent on strain background and when during development *Dmrt1* is deleted. The functions of DMRT1 include control of embryonic germ cell pluripotency and differentiation and proliferation of both germ cells and Sertoli cells.
We now report that deletion of *Dmrt1* in undifferentiated spermatogonia causes these cells to cease spermatogonial development and to prematurely enter meiosis. The presence or absence of DMRT1 therefore determines whether male germ cells undergo mitosis and spermatogonial differentiation or meiosis. We find that DMRT1 acts in spermatogonia to restrict RA responsiveness and also directly represses *Stra8* transcription, thereby preventing meiosis. In vitamin A deficient animals (lacking RA) wild type germ cells arrest as undifferentiated spermatogonia, whereas *Dmrt1* mutant germ cells can initiate meiosis but cannot complete it. DMRT1 also promotes spermatogonial development, and directly activates transcription of the spermatogonial differentiation factor *Sohlh1*. Germ cell specific loss of DMRT1 also disrupts cyclical gene expression in Sertoli cells.
Our results indicate that DMRT1 allows abundant and continuous production of sperm by coordinating spermatogonial development and mitotic amplification with meiosis in the adult testis.

TRANSMISSION AND ANTAGONISM OF GERMLINE FATE IN *C. ELEGANS*

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Perpetuation of the *C. elegans* germ line from generation to generation depends on the MES proteins, named for their maternal-effect sterile phenotype. MES-2, MES-3, and MES-6 form the worm version of the Polycomb Repressive Complex 2 and methylate histone H3 on Lys 27 (H3K27), a modification associated with repression of gene expression. MES-4 is a homolog of the mammalian NSD proteins and methylates H3K36, a modification associated with active gene expression. Numerous studies have linked MES-2/3/6 and MES-4 to global repression of gene expression from the X chromosomes in the germ line, but have also suggested that MES-4 serves an additional critical role in early primordial germ cells (PGCs). Recent chromatin immunoprecipitation analysis of the distribution of MES-4, RNA Polymerase II, and H3K36 methyl marks across the genome in early embryos suggests that MES-4 serves a "memory" role. In contrast to previously studied H3K36 methyltransferases, which are targeted to genes by association with Pol II, MES-4 can associate with genes in a Pol II-independent manner. The genes that MES-4 binds and methylates in embryos are genes that were previously expressed in the maternal germline, many of which are no longer expressed in embryos. These and other findings suggest that MES-4 transmits the memory of gene expression in the parental germ line to offspring and ensures the ability of the PGCs to execute a proper germline program. Our findings raise the question of how somatic cells in the embryo, which also inherit MES-4 marking of germline genes, avoid following a germline fate. The synMuv B chromatin regulators play a key role, as their loss causes somatic cells to misexpress numerous germline-specific genes and causes larvae grown at elevated temperature to arrest. Concomitant loss of maternal MES-4, or loss of MES-2/3/6, suppresses the germline potential of somatic cells and larval arrest. Thus, the MES and synMuv B proteins serve opposing roles in the germ-soma decision.

A NON-CANONICAL ULTRASTRUCTURE PACKAGES DNA IN *C. ELEGANS* OOCYTES

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Chromatin dynamics are closely intertwined with many aspects of germ cell development. Remarkably, oocytes are capable of expressing a specific set of genes as chromatin reaches the highest level of condensation. To characterize the underlying structures of genomic DNA in oocyte chromatin, we used an in situ genome-wide mapping approach using micrococcal nuclease (MNase), high throughput DNA sequencing, and *C. elegans* as a model system.

Unexpectedly, when oocyte lysate was lightly treated with MNase, the resulting DNA fragments exhibit an extensive ladder pattern with a distinct step size of 10 nucleotides on a gel. Such patterns are absent in control samples, including naked worm DNA and chromatin from other stages of worms.

We have taken two approaches to further characterize the oocyte chromatin. In a genomic approach, we massively sequenced the 10-bp ladder-like oocyte DNA fragments and generated a high-resolution genome-wide map of the corresponding MNase-sensitive sites. In parallel, we performed chromatography purification of the oocyte chromatin fragments. Our results indicate that a large fraction of the oocyte chromatin is biochemically distinct from the canonical nucleosome-based chromatin. This novel chromatin structure corresponds to oocyte gene expression and is highly influenced by the underline DNA sequence.

REGULATING THE GERMLINE EPIGENOME ACROSS GENERATIONS IN C. ELEGANS.

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As germ cells traverse generations, they carry both genetic and epigenetic information that can inform the developmental programs of each successive generation. Conversely, developmental programs, once engaged, can impose informative changes to the epigenome in any lineage including, potentially, the germ line. Epigenetic changes can stably impact pluripotency, so alterations in epigenetic information imposed during germ cell development (e.g., gametogenesis) in one generation could affect germ line totipotency, and hence fertility and development, in the next. It is presumably for this reason that epigenetic reprogramming mechanisms are active in both the zygote and during primordial germ cell specification: they are probably required to re-establish an epigenome that is compatible with pluripotency after the germ line encounters developmental programming mechanisms at different stages in its cycle.

We are using C. elegans to examine the processes that define and maintain the totipotent epigenome during the germline cycle. We have shown that histone methylation, like DNA methylation, can be established differentially and heritably maintained across generations. Our recent studies indicate that histone H3 lysine 4 and lysine 36 methylation (H3K4me and H3K36me, respectively) can be established during germline transcription, yet can also be maintained independently of transcription during different germ line stages. We have also shown that reprogramming mechanisms that target H3K4me are essential for maintaining germline immortality, and thus seem to be required to re-establish an epigenetic "ground state" at each generation to prevent heritable, trans-generational accumulation of aberrant epigenetic information.

We propose that the germline epigenome is established and maintained by a reiterative cycle of transcription-dependent and transcription-independent mechanisms, and that this information is filtered through both selection and erasure mechanisms that determine what information is appropriate to pass down the generations. How the system discriminates between what is erased and what is maintained, and the mechanisms that regulate these decisions, are questions we are beginning to explore. Our recent data on these questions will be presented.

LOSS OF TELOMERE IDENTITY OF THE PATERNAL GENOME IS RESPONSIBLE FOR THE PATERNAL EFFECT LETHALITY IN DROSOPHILA *MALE STERILE K81* MUTANTS

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During spermatogenesis from flies to men, chromatin undergoes massive remodeling that involves replacement of the bulk of chromosomal proteins with protamine and similar proteins. This creates a challenge for re-establishing the epigenetic landscape on the paternal genome upon fertilization. It is widely believed that selective retention of chromosomal proteins in mature sperms, such as centromeric histones, demarcates epigenetic landmarks on the paternal genome. It has been difficult to formally test this hypothesis as loss of these proteins disrupts somatic development. In addition, a mark has not been identified for the paternal telomeres. Here we show that the previously identified MS(3)K81 (K81) protein marks Drosophila telomeres during spermatogenesis and its loss specifically destabilizes the paternal genome during post fertilization development. The *ms(3)k81* (*k81*) gene was a recent duplication of the *hiphop* gene, which encodes a telomere specific capping protein that we identified recently. While HipHop protects telomeres in somatic cells, K81 is produced exclusively in males and localized at telomeres of post-mitotic cells, including mature sperms. Loss of K81 does not impair germline development as *k81*-mutant males produce motile sperms that are able to fertilize wild type eggs. In these embryos, however, the inability of the maternal supplies to re-establish a protective cap for the paternal telomeres leads to end-to-end fusion. These fusions hinder the segregation of the paternal genome during the first zygotic division, resulting in haploid embryos with only maternal chromosomes. The functional divergence between *hiphop* and *k81* manifests not only in their expression patterns but also in the function of the proteins that they encode. We conducted mutant-rescuing experiments that involved swapping of the two coding regions. We show that K81 can replace HipHop to protect telomeres in the soma while HipHop cannot replace K81 in the germline to specify telomere identity, since HipHop expressed in the male germline is removed from telomeric chromatin during sperm maturation. We identified a short stretch of amino acid residues that are essential for K81 to survive this chromatin remodeling process. Our study demonstrates the importance of paternal imprints on sperm chromatin for the functional re-establishment of the paternal genome upon fertilization. It also illustrates one of the mechanisms by which telomere functions drive genome evolution.

GENE PACKAGING AND CHROMATIN MODIFICATIONS IN ZEBRAFISH GERM CELLS

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A key issue in early development is the gene packaging assymetry of the paternal and maternal gametes and its resolution in the embryo. Although much of the genome is packaged in protamine in mature human sperm, genes of importance for embryo development (i.e. transcription factors) lack DNA methylation and bear nucleosomes with distinctive histone modifications, suggesting the specialized packaging of these developmental genes in the germline. Here, we explored the tractable zebrafish model and found conceptual conservation as well as several new features. Biochemical and mass spectrometric approaches reveal the zebrafish sperm genome packaged in nucleosomes and histone variants (and not protamine), and we find linker histones high and H4K16ac absent - key factors which may contribute to genome condensation. We examined several activating (H3K4me2/3, H3K14ac, H2AFV) and repressing (H3K27me3, H3K36me3, H3K9me3, hypoacetylation) modifications/compositions genome-wide, and find developmental genes packaged in large blocks of chromatin with coincident activating and repressing marks and DNA hypomethylation, revealing complex 'multivalent' chromatin. Notably, genes that acquire DNA methylation in the soma (muscle) are enriched in transcription factors for alternative cell fates. Remarkably, we find H3K36me3 located in 'silent' developmental gene promoters, and not present at the 3' ends of coding regions of genes heavily transcribed during sperm maturation, suggesting different rules for H3K36me3 in the germline and soma. We also reveal the chromatin patterns of transposons, rDNA, and tRNAs. Finally, high levels of H3K4me3 and H3K14ac in sperm are correlated with genes activated in embryos prior to the mid-blastula transition (MBT), whereas multivalent genes are correlated with activation at or after MBT. Taken together, gene sets with particular functions in the embryo are packaged by distinctive types of complex and often atypical chromatin in sperm.

POLYCOMB FUNCTION DURING OOGENESIS IS REQUIRED FOR MOUSE EARLY EMBRYONIC DEVELOPMENT.

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In mammals, fusion of a highly differentiated oocyte and spermatozoon leads to the formation of a totipotent early pre-implantation embryo. Acquisition of totipotency concurs with remodeling of chromatin states of parental genomes ("epigenetic reprogramming"), changes in maternally contributed transcriptome and proteome, and zygotic genome activation. Genomes of mature germ cells are more proficient in supporting embryonic development than those of somatic cells. It is currently unknown whether transgenerational inheritance of chromatin states present in mature gametes underlies the efficacy of early embryonic development after natural conception. Here, we show that Ring1a and Rnf2, two core components of the Polycomb Repressive Complex 1 (PRC1), serve redundant gene regulatory functions during oogenesis that are required to support embryonic development beyond the two-cell stage. Numerous developmental regulatory genes that are established Polycomb targets in various somatic cell types are de-repressed in Ring1/Rnf2 double mutant (dm) fully grown germinal vesicle (GV) oocytes. Translation of tested aberrant maternal transcripts is, however, delayed until after fertilization. Exchange of maternal pro-nuclei between control and Ring1/Rnf2 maternally dm early zygotes demonstrated an essential role for Ring1 and Rnf2 during oogenesis in defining cytoplasmic and nuclear maternal contributions that are both essential for proper initiation of embryonic development. A large number of genes up-regulated in Ring1/Rnf2 dm GV oocytes harbor PRC2-mediated histone H3 lysine 27 trimethylation (H3K27me3) in spermatozoa and in embryonic stem cells, and are repressed during normal oogenesis and early embryogenesis. These data strongly support the model that Polycomb acts in the female and male germline and during early embryogenesis to silence differentiation inducing genes, thereby sustaining developmental potential across generations.

TRANSCRIPTIONAL REGULATION OF SPERMATOGONIAL STEM CELL SELF RENEWAL

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TAF4b is a gonadal-enriched component of the well conserved general transcription factor complex TFIID and is required for oogenesis and spermatogenesis in the mouse. Male mice that lack TAF4b complete a limited amount of spermatogenic differentiation but fail to maintain spermatogenesis throughout adult life. In mammals, the ability to maintain spermatogenesis is largely the function of spermatogonial stem cells (SSCs) and their associated niche that provides continuous production of spermatogenic progenitors throughout most of adult life. Successful germ cell transplantation assays into adult TAF4b-deficient host testes previously demonstrated that TAF4b is apparently dispensable for providing essential components of the adult SSC niche. Here, we test the hypothesis that TAF4b regulates a germ cell-autonomous program of gene transcription required for the development of self-renewing SSCs in early postnatal life required for full spermatogenic maintenance in the adult. While more is known about the signaling pathways required to maintain the self renewing properties of the mammalian SSC, little is known about the potential transcriptional regulators that help establish the program of SSC gene transcription that is required for establishing the self renewing SSC lineage.

To begin to understand the role of TAF4b in promoting SSC self-renewal, we have compared gene expression programs in an SSC-enriched fraction of the early postnatal TAF4b-deficient testis compared with a similar SSC-enriched fraction of matched wild type littermates. This analysis revealed a number of potential regulators of SSC renewal to be underrepresented in the TAF4b-deficient enriched SSCs including Gfrα1. To extend these studies, we utilized real time RT-PCR to assay the relative levels of multiple components of the GDNF signaling pathway and its known target genes and observe widespread disruption of this pathway in the TAF4b-deficient testes. GDNF signaling through c-Ret and Gfrα1 co-receptors is known to be critical for SSC self renewal and its disruption in the context of the TAF4b-deficient testes could partially account for the disruption in spermatogenic maintenance in this mutant mouse line. To test the functionality of TAF4b-deficient SSCs, we have determined that wild type, but not TAF4b-deficient SSCs, can repopulate the testis of a germ cell deficient host via germ cell transplantation. In combination with these gene expression studies, functional assays of SSC repopulation indicate that TAF4b is a critical transcription regulator of an SSC self renewal program in the mammalian testis. Consistent with this role, we, and others, detect high levels of Taf4b expression in mouse SSCs. Together, these studies are beginning to reveal how a specialized component of the basal transcription apparatus has evolved the exquisite function of regulating SSC renewal, in part through regulation of the GDNF signaling pathway, in the mammalian testis.

GERMLINE-SPECIFIC EPIGENETIC REPROGRAMMING IN CLONED AND ICSI MICE

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The use of either somatic cell nuclear transfer (SCNT) or assisted reproductive technology (ART) has been implicated in causing epigenetic abnormalities, and while previous studies have reported aberrations in DNA methylation profiles in SCNT and ART mouse models, the question of whether these epigenetic defects are propagated to subsequent generations thru the ensuing germ line has not been thoroughly investigated. To this end, we analyzed allele-specific DNA methylation and expression of the paternally imprinted gene, H19, in somatic tissues and germ cells of mice produced by SCNT or intracytoplasmic sperm injection (ICSI), a type of ART, and compared these to those in naturally conceived mice to determine 1) if SCNT or ICSI lead to epigenetic abnormalities in mice produced by these methods in our hands, and 2) if these errors are retained or corrected in the germ line of these same mice by germline-specific epigenetic reprogramming. We observed three results of interest: 1) we detected DNA methylation abnormalities in the H19 gene in somatic tissues of ICSI mice at both 6 and 60 days postpartum (dpp), but did not detect similar anomalies in the SCNT mice, 2) abnormal biallelic expression was detected in liver from these same ICSI mice at 6 dpp but not at 60 dpp, 3) we observed less extensive DNA methylation on the maternal allele of the H19 gene in male germ cells from 6 dpp ICSI mice than in naturally conceived controls, however by 60 dpp the ICSI mice showed complete, biallelic, germline-specific epigenetic reprogramming of the H19 gene in male germ cells. A unifying hypothesis that is consistent with all of these results is that exposure of the maternal gametic genome to endocrine stimulation can result in the induction of epigenetic abnormalities that can be transmitted to offspring. Such epigenetic defects appear to be sustained in somatic tissues of these offspring, but are corrected in germ cells by germline-specific reprogramming. Our analysis of SCNT mice provides a uniquely valuable control for this conclusion because although these mice were also derived from oocytes that had been exposed to endocrine stimulation, the genomes of these oocytes were then removed as part of the SCNT process and replaced with genomes from somatic donor cells that had not been subjected to endocrine stimulation. This suggests that endocrine stimulation to enhance folliculogenesis may lead to an increased incidence of epigenetic errors in the maternal germline, but also indicates that epigenetic errors inherited and maintained in somatic tissues of ICSI mice are typically corrected by epigenetic reprogramming in the germ line of these same animals.

C. ELEGANS DICER INTERACTS WITH THE P-GRANULE COMPONENT GLH-1.

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In *Caenorhabditis elegans*, maintenance of a productive germline is dependent on the germline RNA helicase, GLH-1, an integral component of the germline-specific P granules that surround the nuclei of germline progenitor cells, germline stem cells, and developing oocytes and sperm. Our laboratory has discovered that GLH-1 and the ribo-endonuclease Dicer-1 (DCR-1) are interdependent both by genetic and biochemical analyses. These two proteins bind each other and both localize to P granules at the pachytene stage of oogenesis when many maternal RNAs are processed and stored. Both GLH-1 protein and mRNA levels are reduced in the *dcr-1(ok247)* mutant background; conversely, a reduction of DCR-1 protein is observed in the *glh-1(gk100)* deletion strain. In addition, evidence indicates levels of DCR-1 protein, like that of GLH-1, are regulated by proteosomal degradation, likely targeted by the Jun N-terminal kinase KGB-1. In adult germ cells DCR-1 is located in uniformly-distributed, small puncta throughout the cytoplasm, as well as in close proximity to the inner side of the nuclear pores, and to P granules. In arrested oocytes, GLH-1 and DCR-1 re-localize to cortically-distributed RNP granules, and are necessary to recruit other components to these complexes. We predict the GLH-1/DCR-1 complex may function in the transport, deposition, or regulation of maternally-transcribed mRNAs and their associated miRNAs.

A NOVEL POST-DIFFERENTIATION ROLE FOR NOTCH SIGNALING IN *C. ELEGANS* OVULATION

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Signal transduction pathways that govern cell fate decisions and influence stem cell behavior often have multiple roles at the same anatomical loci. Notch signaling has been studied in many contexts, most of which involve cell fate specification during development. Fewer examples can be found in which Notch signaling is required for post-differentiation processes. Here we present our findings suggesting that Notch signaling plays an important role in *C. elegans* ovulation – a highly regulated, complex process that involves multiple germline-soma signaling events that coordinate meiotic maturation, sheath contraction, spermathecal dilation, and fertilization. Previously we showed that APX-1, a conserved ligand for Notch family receptors, acts in the proximal sheath of the *C. elegans* gonad as a latent proliferation-promoting signal that maintains proximal germline tumors in a variety of mutant backgrounds. Additionally, we showed that in this "latent niche" role, APX-1 acts through the Notch receptor GLP-1 and is required continuously to maintain tumors. The "latent niche" role is a consequence of abnormal cell-cell interactions between the sheath and germline stem/progenitor cells. However, a normal functional role for Notch signaling in the proximal sheath had not been previously characterized. To begin to address the normal role of Notch signaling in the proximal gonad, we are focusing on *apx-1*. Upon reduction of *apx-1* activity, we observed endomitotic oocyte nuclei (Emo). Time-lapse microscopy confirmed defects in ovulation that likely account for the Emo phenotype. Moreover, the ovulation defects are soma-dependent. Finally, we determined that LIN-12 is the relevant Notch receptor for this role. Proper ovulation depends on correct somatic cell fate specification during development, as well as a host of signaling events that occur with each ovulation. We examined a variety of cell-fate markers and were surprised to find no alteration in cell fate specification with reduced Notch signaling. Additionally, we found that signaling is required continuously throughout adulthood to maintain proper ovulation. Upon removal of either APX-1 or LIN-12 in normally-ovulating adults by RNAi, ovulation defects ensued. Together these results suggest a novel post-differentiation role for Notch signaling in ovulation.

MICRORNA SIGNALING IN EARLY MALE GERM CELLS

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Carcinomas in Situ cells, the precursor state of testicular germ cell tumours, have been identified as dysfunctional gonocytes. Therefore differentiation process of gonocytes into spermatogonia is of great interest due to the continual rise in the rate of testicular cancer in the developed world. MicroRNAs are short regulatory non coding RNA molecules that bind to the 3' untranslated region of several mRNA targets to control translation and therefore control the abundance of many different protein molecules within a cell. Aberrant expression of miRNA is linked to many diseases and developmental abnormalities.

Gonocytes from post natal day 1 and spermatogonia from day 7-9 mice were enriched by 2-4% BSA gradient sedimentation. Total RNA including microRNA was extracted and analyzed in by Illumina miRNA microarray. Total RNA was also reverse transcribed using specific primers and analyzed by qPCR. Three biological replicates were performed in both the microarray and qPCR experiments.

Bioinformatic analysis with SAM (Significance analysis of Microarrays) identified seven significantly different miRNA molecules expressed in spermatogonia and gonocytes.

qPCR analysis confirmed two miRNAs were significantly up regulated in spermatogonia (743a, 463*) and three miRNAs were significantly down regulated in spermatogonia (293, 290-5p, 291a-5p) when compared to gonocytes.

Several miRNA molecules were selected for further study (293, 290-5p, 136, and 146a) and overexpression assays in first P19 cells then spermatogonia will help determine their function. In the future the role of these miRNA molecules in seminoma will be analysed using over-expression within a seminoma cell line. It is hypothesized that these miRNA molecules control genes involved in male development and differentiation such as stella, nanog and oct3/4 and that these molecules may also play a role in tumour development

In conclusion miRNA expression is significantly different between gonocytes and spermatogonia and influences their differentiation and developmental course.

DO MALE GERM CELLS ALSO USE TRANSCRIPTION TO INDUCE DNA METHYLATION AT GENE PROMOTERS?

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In female germ cells, transcription of coding RNAs through downstream gene promoters has been shown to induce gene silencing by DNA methylation. This was specifically shown at those imprinted genes that gain methylation in the female germline. However, it is possible that such transcription driven induction of DNA methylation be a mechanism employed routinely by other cell types like somatic cells, and indeed male germ cells. I am interested in understanding what mechanisms the paternal genome uses to drive silencing, and indeed DNA methylation at promoters of paternally methylated imprinted genes; and have looked for evidence of transcription driven methylation at an imprinted locus in the male germline during embryonic development. The maternal and paternal genomes, believed to be locked in a conflict of interests, epigenetically program their respective germlines in preparation for the post-fertilization tussle for maternal resources by silencing certain genes. It is interesting that the male and female germlines may employ similar mechanisms like transcription driven DNA methylation of gene promoters to achieve conflicting objectives.

JARID-REGULATED GLOBAL HISTONE DEMETHYLATION OF HISTONE H3 LYSINE-4 MODULATES THE GENE EXPRESSION PROGRAM OF GAMETOGENESIS IN *S. CEREVISIAE* TO CONTROL THE TIMING OF SPORE DIFFERENTIATION.

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The ancient and expansive JARID family of histone demethylases controls stem cell differentiation and tumorigenesis in mammals. While *bona fide* histone demethylases of this family are found in budding yeast, their physiological functions have yet to be identified. We have discovered that *JHD2*, an ortholog of mammalian JARID1A/Rbp2, functions to accomplish developmentally programmed global demethylation of histone H3 lysine-4 (H3-K4) during yeast sporulation, with the bulk of demethylation occurring post-meiotically. Using a sensitive cell-biological assay, we determined that Jhd2-demethylation of H3-K4 controls the timing of spore differentiation: Amazingly, both *jhd2*Δ and an enzymatically-dead allele of *jhd2* cause precocious terminal differentiation. Chromatin-modifying enzymes such as Jhd2 have been thought to contribute to gene regulation through their recruitment to specific genes by sequence-recognizing DNA-binding proteins. We found, rather, that Jhd2 regulation of the yeast sporulation program involves global, gene nonspecific regulation: *JHD2* null mutants exhibit global defects in mRNA accumulation occurring during the post-meitoic stages of gametogenesis which indicate that Jhd2 demethylation of H3-3meK4, a chromatin mark often regarded as activating, is required for genes to be fully transcribed. Furthermore, chromosome-wide analysis of H3-3meK4 and H3-2meK4 distribution in sporulating cells show that *jhd2*Δ mutants generally accumulate hypermethylated nucleosomes around transcription start sites. These studies reveal that repressive histone H3K4 methylation effectively constitutes a global "volume knob" for JARID-mediated control of developmental gene expression. Finally, our additional genetic analyses suggest that inter-organelle signaling between the mitochondria and the nucleus *via* Jhd2 may underlie spore differentiation. Indeed, consideration of JARID1 function during human stem cell development lends support to the idea that such phenomenon may be fundamental to eukaryotic differentiation. These hypotheses will be discussed.

PRIMORDIAL GERM CELL LOCALIZATION WITHIN A NOVEL STEM CELL NICHE OF THE MURINE ALLANTOIS

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Prior to their migration through the hindgut and colonization of the gonads, the primordial germ cells (PGCs) localize to the posterior region of the mouse conceptus. This posterior site encompasses the embryonic posterior primitive streak and the allantois, an extraembryonic tissue that gives rise to the umbilical cord. Intriguingly, PGC localization to the posterior region coincides with the presence of the Allantoic Core Domain (ACD), a putative stem cell reservoir in the proximal midline of the allantois. The ACD is required for the allantois to elongate and form the umbilical cord. Because it is unclear whether PGCs are part of, or independent of, the ACD, we sought to clarify the relationship between the ACD and PGCs. A spatiotemporal localization study of Stella, which is presumed to mark definitive PGCs, revealed that the domain of Stella localization extends from the posterior primitive streak into the ACD. Intriguingly, in the allantois, the majority of Stella-positive cells localized to the proximal ACD. Synchronous (same-stage) orthotopic (same-site) grafts of allantoic subregions revealed that the proximal ACD, but not the distal ACD or distal allantois, contributed Stella-positive cells to hindgut. Finally, microsurgical removal of the allantois and associated endoderm reduced, but did not eliminate, the Stella-positive population in the hindgut. Together, these results indicate that the proximal ACD contributes to, but is not the exclusive source of, Stella-positive PGCs in the hindgut.

RET SIGNALLING IS REQUIRED FOR FETAL GERM CELL SURVIVAL

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Germ cells are committed to the male or female pathway during fetal development and differentiate into sperm and oocytes later in life. During fetal development male germ cells proliferate extensively until they enter a period of quiescence. Previously we have performed detailed analyses of male germ cell entry into mitotic arrest during embryonic day (E) 13.0-E14.5. Our analyses have shown that germ cell mitotic arrest involves the upregulation of the cell cycle inhibitors $p27^{KIP1}$, $p15^{INK4b}$ and the activation of RB. However, the signalling events that promote the entry of male germ cells into G1/G0 mitotic arrest are unknown. One signalling candidate that is upregulated specifically in the developing male gonad during this period is GDNF. GDNF signalling is mediated through its receptor RET and has been implicated in teratoma formation. By analysing *Ret* -/- and *Gdnf* -/- mice we hope to gain insight into the role of GDNF/RET signalling during male germ cell development. We show that there is significant loss of germ cells in E15.5 *Ret* -/- mice, although lack of RET function does not hinder mitotic arrest of the remaining MVH positive male germ cells. Furthermore the subpopulation of *Ret* -/- germ cells that no longer express MVH, abnormally express the cell cycle regulator $p27^{KIP1}$ and the proliferation marker KI-67. These results identify a novel function for RET in germ cell survival.

ROLE OF POLY (ADP-RIBOSE) POLYMERASE1 (PARP1) ENZYMATIC ACTIVITY IN MALE GAMETES

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Maintaining the integrity of sperm DNA is vital to reproduction and male fertility. PARP1 and PARP2 have diverse roles in spermatogenesis and in ejaculated sperm. Expression of several PARPs is associated with sperm maturity in fertile men [1] indicating important functions of PARP proteins and/or its enzymatic activity in spermatogenesis. However, PARP1 knock-out mice show normal reproductivity [2], and mice carrying caspase-resistent PARP1 (PARP1 $DEVD_{D214N}$ knock-in) also reproduce normally [3]. However, PARP inhibitor causes a defective DNA repair in spermatids and spermatocytes [4], suggesting that the PARP activity is important for maintaining sperm DNA integrity. To investigate which in vivo function of PARP1 requires enzymatic activity and which relies on enzymatic activity independent PARP1 interactions, we have generated two mouse models with a point mutation in the catalytic domain of PARP1 ($PARP1^{D993A}$ and $PARP1^{L751A}$). The biochemical analysis revealed that their PARP1 enzymatic activity is reduced by ~20% and ~80%, respectively. In contrast to the use of PARP inhibitors or PARP1 complete knockout mice, this approach allows the investigation of PARP1 enzymatic activity without simultaneous inhibition of other PARP family members, but still containing endogenous PARP1 with modulated enzyme activity. The $PARP1^{L751A}$ male mice show a severe infertility phenotype, whereas $PARP1^{D993A}$ mice produce normally. It seems that the certain threshold of poly(ADP-ribose) forming activity, but not PARP1 protein per se, is critical for spermatogenesis.

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SOX9b FUNCTION IN GERMLINE STEM CELL NICHE IN THE OVARY OF TELEOST FISH, MEDAKA

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Sry-related HMG box transcription factor, *sox9* is upregulated only in male fetal gonad during sex differentiation of the gonad and is required and sufficient for testis differentiation in mammals. In contrast, *sox9b*, medaka orthologue of *sox9*, is expressed in both female and male gonads from the gonadal primordia to adult ovaries and testes (Nakamura et al., 2008). Recently we have revealed germline stem cells are present in the *sox9b*-expressing cord-like structure, termed ovarian cords, in the medaka adult ovary, indicating the unknown function of *sox9b* in the medaka gonad (Nakamura et al., 2010).
Here we characterized the *sox9b* tilling mutant medaka to explore the *sox9b* functions in the gonadal differentiation and the regulation of germline stem cells. In adult heterozygous *sox9b* mutant medaka, germ cells are lost and female to male sex reversal occurs. We examined the proliferation of germ cells, the cellular identity, the morphology and the sexual states of mutant gonads by checking marker expressions together with the chimeric analysis using wildtype and mutant medaka, indicating that novel functions of *sox9b* on the germline in the ovarian cords. Our analysis also suggests that *sox9b* is not directly involved in the sex differentiation.

RNAi SPREADING MUTANTS *RSD-2* AND *RSD-6* ARE DEFICIENT FOR GERM CELL IMMORTALITY

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Germ cells represent canonical stem cells that possess the remarkable quality of being able to proliferate from one generation to the next, indefinitely, free of replicative damage. *mortal germline (mrt)* mutants initially display normal levels of fertility, but become progressively sterile when grown for multiple generations. Of 16 *mrt* mutants identified in a pilot EMS screen (Ahmed and Hodgkin, 2000), most were temperature-sensitive and only became sterile when grown at 25oC. Of the ts mutants, two were resistant to RNA interference (RNAi) feeding constructs targeting embryonic lethal genes: *1c* and *10i*. Genetic mapping experiments, complementation tests for RNAi resistance, and DNA sequencing revealed that *1c* and *10i* contain mutations in *rsd-6* and *rsd-2*, respectively. Independently isolated alleles of *rsd-6* and *rsd-2* confer progressive sterility when propagated at 25oC, and complementation tests confirmed that these genes are required for germ cell immortality. *rsd-6* and *rsd-2* have been reported to be deficient for spreading of dsRNA-mediated RNAi from somatic cells to germ cells (Tijsterman et al., 2004). Our results indicate that RSD-6 expression in germ cells may be sufficient to promote germ cell immortality or RNAi. Germ line morphology of sterile *rsd-2* or *rsd-6* mutants indicates a stochastic developmental response to the heritable damage transmitted by these strains at restrictive temperature. We are investigating the mechanism by which *rsd-2* and *-6* promote germ cell immortality.

UNDER STRESS CONDITIONS TIA-1.2 IS REQUIRED TO INDUCE APOPTOSIS AND THE FORMATION OF GRANULES IN THE *C. ELEGANS* GONAD

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Germ cell apoptosis is an evolutionary conserved process important to maintain the homeostasis and quality of oocytes. In *C. elegans*, half of germ cells are eliminated during oogenesis by an unknown mechanism, which can be also triggered by stress. In an RNAi screening to identify proteins important for stress-induced apoptosis, we found TIA1.2 (C18A3.5); one of the three TIA-1/TIAR homologs in *C. elegans*. TIA-1 and TIAR are RNA binding proteins that promote the assembly of stress granules, and induce apoptosis in mammals. Stress granules are termporally formed RNP complexes where mRNAs are stored and protected. We found that TIA-1.2 is a cytoplasmic protein that is expressed in the soma and the germline constituvely. *tia-1.2(tm361)* animals are shorter, move slower, and have less offspring than wild type due to defects during oogenesis, spermatogenesis and embryogenesis. We found that TIA-1.2 is required to induce germ cell apoptosis in response to several stress conditions. To test if TIA1.2 is important for granules formation under stress conditions, we exposed wild type and *tia-1.2(tm361)* animals to starvation, heat shock and UV light. As a granule marker we used CGH-1; a DEAD box RNA helicase that is expressed diffused in the cytoplasm, in small granules and germ granules in the adult gonad and embryos. When wild type nematodes were exposed to starvation and heat shock, large granules accumulated in the distal core of the gonad. More discrete granules were also observed after UV light treatment. Accumulation of granules was not observed when animals were subjected to stress in the presence of cycloheximide; a drog that disassembles both stress granules and P-bodies in mammals. This suggests that granules formed under stress conditions in the distal core of the gonad are similar to mammalian stress granules and P-bodies. *tia-1.2(tm361)* animals subjected to stress conditions showed CGH-1 in small granules and P granules, however no large core granules were observed suggesting that, like in mammals, this protein is important for granules assembly. The other TIA-1/TIAR related genes in *C. elegans* are not required to induce germ cell apoptosis under stress conditions, but they play a partial role in granules assembly under stress conditions.

CHARACTERIZATION OF THE OOCYTE PHENOTYPE CAUSED BY *MCL-1* DEFICIENCY

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Survival of the female germ line is a carefully monitored physiological process, with several windows of death susceptibility. Many oocytes are created during embryogenesis, but only a small fraction survives to mature and result in developmentally competent zygotes. Thus, death rather than growth is a fate most commonly experienced by the female germline. Interestingly, premature ovarian failure (POF) due to an exhaustion of follicles has not been observed in females with disruption of genes belonging to the anti-apoptotic *Bcl-2* family members analyzed so far. Considering the functional redundancy of the *Bcl-2* family and the significant fraction that are expressed by oocytes, it is possible that several members of this family have to be inactivated in order to attain a POF like phenotype. Additionally, a *Bcl-2* family member governing the survival of follicles has not yet been identified. We propose that *Mcl-1* is the key *Bcl-2* family member, governing female germ line survival. In order to study the role of *Mcl-1* in oocytes, we conditionally deleted this gene via activation of *Cre* expression under the control of the oocyte-specific zona pellucida (*ZP-3*) promoter in a mouse model.

Mcl-1 oocyte-deficient females are capable of producing only two to three litters with a significant decrease in litter size, as they develop premature ovarian failure (POF) around 4 months of age. This affect on fertility was confirmed with histomorphometric analyses of ovarian follicles, showing a sharp reduction in oocyte number (particularly primordial and primary oocytes) as early as 3 weeks of age. At this age, we were able to isolate mature oocytes by superovulation and subject these to various assays to determine mitochondrial endowment and function. Ovulated *Mcl-1*-deficient oocytes are highly susceptible to apoptosis, exhibiting a plethora of mitochondrial defects linked to metabolic output with evidence of mitochondrial depletion (reduction in mitochondrial membrane potential, increases in oxidative stress and decreases in NAD(P)H production). Additionally, TEM imaging revealed an increase in lysosome formation and structural mitochondrial defects, consistent with activation of mitophagy. These findings indicate underlying defects in coupling of mitochondrial and metabolic functions as a cause for oocyte loss, and implicate *Mcl-1* as a key *Bcl-2* family member governing oocyte survival.

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CLONING AND MOLECULAR ANALYSIS OF THE MURINE *CATSPER* 1 PROMOTER, A PROMOTER UPREGULATED BY SOX9.

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Catsper is a calcium channel present exclusively in sperm and crucial for ovocite fertilization. Physiological and biochemical studies have demonstrated that Ca^{++} influx through flagella is driven by Catsper channels. Up to date, the regulation mechanism for its transcription is unknown. The gene *CatSper1* expresses a testis specific 2.6 kb transcript, comprised by 12 exons: the first exon accounting for approximately 45% of the whole CDS. *Catsper1* starts its transcription in the spermatocyte stage, between 18 and 21 days after meiosis and reaches its maximum level during adulthood. We tested for basal transcriptional activity levels, an initial construct of 1.2 kilobases, which included the exonic region +277 in heterologous systems. Unexpectedly, the same promoter region cloned in antisense direction, presented transcriptional activity beyond a sequence not yet annotated in the mouse genome. The deletion that keeps the +23-599 region of the *CatSper1* promoter and eliminates a SRY binding site, showed an increased activity by 6 folds. The shortest deletion (+23-261) still maintained its high transcriptional activity. The sequence of the +23-599 promoter contains two binding sites for SOX9, a transcriptional factor expressed in Sertoli cells. Cell transfection experiments showed that SOX9 expression transactivates the *CatSper1* promoter up to 50 folds. On the contrary, site-directed mutagenesis on each of the SOX9 sites diminished its upregulation; moreover, the double mutation reduced the whole transactivation to basal transcriptional levels even with SOX9 present intrinsically. These results indicate that SOX9 regulates the expression of the murine *CatSper1* promoter in heterologous systems. We hypothesized that in the testicular environment, Sertoli cells might provide transcriptional factors for the transactivation of *CatSper1* promoter during spermatogenesis.

VBH-1, THE *C. ELEGANS* HOMOLOG OF *DROSOPHILA*'S VASA AND BELLE DEAD BOX RNA HELICASES, HAS A ROLE IN STRESS RESPONSE

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Germ cells from several organisms possess specialized cytoplasmic granules made of mRNA and proteins, which are essential for its development and function, known in *C. elegans* as P granules. VBH-1 is the homolog of *Drosophila*'s DEAD box RNA helicases Vasa and Belle. In *C. elegans*, VBH-1 is important for embryogenesis and gametogenesis, and plays an important role in the sperm/oocyte switch in the hermaphrodite gonad. VBH-1 associates constitutively to P granules, and is also found in the cytoplasm of all blastomeres during embryogenesis, and in the male and hermaphrodite gonad. To test the function of different VBH-1 domains on its localization, we divided this protein in three different regions. The amino domain consists of first 151 aa, the helicase domain (336 aa) and carboxyl-terminus domain (last 157 aa). We fused each *vbh-1* region to a *gfp* reporter under the control of a *pie-1* promoter, and introduced each construct into animals by biolistic transformation. We found that the helicase and carboxyl-terminus domains are sufficient for VBH-1 association to P granules. VBH-1 amino domain is not important for its localization to P granules under normal conditions. However, we observed that after heat shock, the amino domain aggregated into foci. Indeed, we observed that all three VBH-1 domains, and full-length VBH-1 protein, associate to large foci under several stress conditions (heat-shock, starvation and sperm depletion). By Western blot analysis, we found that no VBH-1 over expression occurs under stress conditions indicating that granules are the result of VBH-1 aggregation or association with other proteins. CGH-1 is a protein usually found in small cytoplasmic foci of all blastomeres during early embryogenesis and in the adult gonad that might resemble processing bodies. Under stress conditions CGH-1 aggregates into large granules. We found that under stress conditions, VBH-1 foci co-localize with CGH-1. To test a possible role of VBH-1 during stress, we made a survival curve of *vbh-1*(RNAi) animals and found they are more sensitive to heat shock and oxidative stress than mock treated animals. The closest VBH-1 homologue, LAF-1, is also required for stress survival. Specific germline-silencing of each gene individually had no effect on survival of heat-shocked animals suggesting somatic expression is sufficient for a proper response during this stress. The role of Vasa/Belle-family of proteins in stress protection is novel and we are currently studying how they participate in this response.

ANTAGONIZING GERMLINE FATE IN SOMATIC CELLS: OPPOSING ROLES OF THE MES AND SYNMUV B CHROMATIN REGULATORS

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When sperm and egg fuse, the resulting zygote inherits chromatin with germline characteristics. In mammals, erasure of germline character is one of the first steps of embryogenesis. In *Drosophila* and *C. elegans*, germline chromatin character is maintained in all cells as the primordial germ cells (PGCs) are separated from the somatic lineages early in development. As the germ line is set aside, the remaining cells are reprogrammed to acquire a somatic cell fate. Recent studies in *C. elegans* have revealed that mutants in many synMuv B chromatin regulators have defects in this process and display phenotypes that suggest a partial soma-to-germline transformation (Unhavaithaya et al., 2002; Wang et al., 2005). Through microarray analysis and antibody staining, we discovered that numerous germline-specific genes, including genes encoding germ granules and meiosis proteins, are expressed in the somatic cells of synMuv B mutants. Probably as a result of this ectopic expression of germline genes in somatic cells, most synMuv B mutants tested arrest as L1 larvae when grown at high temperature. Indeed, gene mis-regulation in synMuv B mutants is enhanced at elevated temperature, suggesting that synMuv B proteins may function to buffer gene expression in response to changes in environmental conditions such as temperature. Both ectopic expression of germline proteins and high temperature arrest are suppressed by loss of the MES regulators of germline chromatin. Based on phenotype analysis and tissue-specific depletion and addition of synMuv B function, we determined that the tissue responsible for arrest is the intestine. Interestingly, the intestine and germline progenitor cells are developmentally similar – they arise from sister blastomeres, remain in close proximity during embryogenesis, undergo relatively few embryonic divisions, and clonally generate entire tissues. We hypothesize that synMuv B proteins are required to antagonize the action of MES proteins in somatic cells in order to ensure the erasure of germline chromatin character during embryogenesis. This role of the synMuv B proteins is especially important in the intestine and at elevated temperatures.

FUNCTIONAL DISSECTION OF THE "NON-PUF" PART OF PUF PROTEINS

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Members of PUF family are essential for stem cell proliferation in diverse organisms. These proteins are characterized by the presence of a conserved RNA-binding motif called the PUF domain. The PUF domain has been thought to be essential for the translational control of target mRNAs. In addition to the PUF domain, most members of this family in various organisms contain a large N terminal region. However, the functional importance of this N terminal region is poorly understood. We recently found that the C. elegans PUF protein PUF-8, which is essential for several aspects of germ cell development, functions in nuclear mRNA processing/export. To explore the underlying mechanism, we screened for potential interacting proteins using yeast two-hybrid assay. This screen identified several proteins involved in mRNA processing/export, including members of the nuclear pore complex that are essential for mRNA export. To determine the region critical for PUF-8's interaction with these proteins, we tested various deletions of PUF-8 in the above assay. While the PUF domain is dispensable, the N-terminal domain is necessary and sufficient, for the protein-protein interactions. Our results suggest that the PUF proteins may have two distinct functional domains: the conserved PUF domain binds the target mRNA and the N-terminal domain interacts with proteins involved in different processes such as splicing, nuclear export and translational regulation. Thus, the N-terminal domain may confer functional specificity to individual PUF proteins.

GERMLINE STEM CELLS DIFFERENTIATION REQUIRES TRANSCRIPTIONAL SILENCING

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Germline stem cells (GSCs) have been known to require transcriptional silencing of differentiation factors to maintain their stemness. Here we show that, counterintuitively, transcriptional silencing mediated by histone demethylase (S*u(var)3-3/dLsd1*), and histone methyltransferase (*egg/dSETDB1*) is also required during Drosophila oogenesis for the differentiation of stem cells into gametes. This transcriptional silencing occurs in the progeny of GSCs as evidenced by the movement of Su(var)3-3/dLsd1 from the cytoplasm of GSCs to the nucleus of their progeny and the formation of repressive marks there. Intriguingly, we identify major piRNA clusters as the primary targets of this transcriptional repression. We show that sequential action of transcriptional repressors at these clusters is required for up-regulating piRNA production and down-regulating retrotransposons to allow differentiation to proceed. Our discovery of a novel step during GSC differentiation involving epigenetic control of retrotransposon transcription and piRNA production provides new insight into the exquisite control required to properly exit the stem cell fate.

THE PLURIPOTENCY ASSOCIATED *TEX19.1* GENE IS REQUIRED FOR GENETIC AND CHROMOSOMAL STABILITY IN MOUSE GERM CELLS

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In mammals germ cells transmit genetic information to successive generations in a genetically and chromosomally stable manner. *Tex19.1* is a mammal-specific gene whose expression is mainly restricted to germ cells and pluripotent cells. To investigate the function of Tex19.1 we have generated *Tex19.1*$^{-/-}$ knockout mice. *Tex19.1*$^{-/-}$ males are typically infertile, and exhibit increased levels of cell death during meiosis. Around half the *Tex19.1*$^{-/-}$ pachytene spermatocytes have defects in meiotic chromosome synapsis and retain early recombination markers on their asynapsed chromosomes. Furthermore, most *Tex19.1*$^{-/-}$ spermatocytes that reach the first meiotic metaphase contain univalent chromosomes. Thus Tex19.1 is required for faithful chromosome segregation during male meiosis. At the molecular level Tex19.1 promotes suppression of retrotransposons and germ cells accumulate retrotransposon RNA in *Tex19.1*$^{-/-}$ testes. Increased retrotransposition, and the associated increase in DNA damage, may be responsible for some aspects of the *Tex19.1*$^{-/-}$ male phenotype. We are currently investigating whether derepression of retrotransposons in *Tex19.1*$^{-/-}$ female germ cells and pluripotent cells is associated with the female subfertility and embryonic lethality seen in *Tex19.1*$^{-/-}$ mice. Our results suggest that Tex19.1 promotes genetic and chromosomal stability through successive generations by protecting germ cell and pluripotent cells from the mutagenic activity of transposable genetic elements.

HAPLOID CELLS DIFFERENTIATION FROM PLURIPOTENT CELL LINES BY GENETIC OVEREXPRESSING SYSTEM

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Ten to fifteen percent of couples are infertile because of problems linked to the production of few or no oocytes or sperm. Yet our understanding of human germ cell development is poor, at least in part due to inaccessibility of early developmental stages to molecular genetic studies. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) may provide the necessary human genetic system to study germ line development and ultimately treat infertility associated with reproductive aging, premature ovarian failure, and/or poor oocyte and sperm quality. These pluripotent cells can form all three embryonic germ layers, endoderm, mesoderm and ectoderm, but also germ cells. In this study we tested the overexpression effect of the germ cell related genes DAZ, DAZL, BOULE and VASA during the spontaneous differentiation of pluripotent cells. We observed that both hESCs and iPSCs spontaneously differentiated to primordial germ cells (PGCs) and also formed meiotic and post-meiotic cells only when were subjected to the overexpression system. These results suggest that DAZ, DAZL and BOULE genes play a critical role in meiosis regulation as previously described, but also VASA overexpression and subsequent spontaneous differentiation resulted in the formation of haploid cells. These data may provide a useful model for basic human molecular genetic studies in germ line formation and regulation for future clinical studies of potential novel therapeutic applications.

IDENTIFICATION OF A PRENYLATED GERM CELL CHEMOATTRACTANT IN DROSOPHILA

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As in most organisms, Drosophila germ cells migrating to the gonad through different tissues are oriented by repellent and attractive cues expressed along their path. The last step of migration is guided by the enzyme Hmgcr (HMGCo-A reductase) that controls a rate-limiting step for the production of prenyl moieties. In S. cerevisiae, the small peptide a-factor is produced from a precursor, prenylated and then exported by an ABC transporter, through an unconventional secretory pathway, to guide the fusion of mating yeast during sexual reproduction. Interestingly, we found by genetic analysis that the same peptide export and prenylation pathway utilized in yeast is also required in the mesoderm in Drosophila for germ cell association with the Somatic Gonadal Precursors (SGP). We extended these results by developing a new transwell migration assay that makes use of fluorescence-activated cell sorting (FACS) sorted GFP positive germ cells, placed on a top filter and assess their migration toward conditioned media from Kc cells on the bottom well. Using this assay we have shown that germ cells migrate towards a diffusible attractant and that this is dependent on hmgcr and the ABC transporter mdr49 expression in secreting cells. The identification of the prenylated germ cell attractant exported by Mdr49 is crucial for further understanding of this novel chemoattraction mechanism. To this aim, we have set up new knock down conditions in Kc cells that allow large-scale experimentation. Upon statin treatment prenylation is reduced, active peptide production is blocked and germ cells do not migrate in the transwell assay. These conditions are now being used for chemoattractant identification by mass spectrometry. To confirm that the proteins of interest are prenylated we are using a prenyl chemical analogue to detect secreted prenylated peptides. Candidates will subsequently be tested in the transwell migration assay. The results of this approach will be cross-referenced with prenylated candidates identified in a bioinformatics search of the Drosophila genome

TRACKING THE DIFFERENTIATION OF SPERMATOGONIA.

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Spermatogenesis is an ongoing process of production at the base of which exist cells capable of self-renewal. Current cell purification methods allow for the enrichment of spermatogonial stem cells (SSCs). The greatest enrichment is obtained when a population of spermatogonia are bound to laminin, thus implicating the laminin-binding integrin, α6β1. It has also become clear that the differentiation process is not irreversible. As such cells that can no longer be considered SSCs can revert to that phenotype under appropriate circumstances, such as an empty niche. Such conditions are necessary for germ cell transplantation. Therefore any estimate of SSC number based on transplantation is necessarily an over estimate. Furthermore this approach is cumbersome for high throughput screening of a population that, by definition, is small.

To improve the efficiency of SSC isolation we have utilised methods that target the surface of the cell. Phage display technology was used and the epitope-binding fragments identified were subsequently expressed as recombinant proteins. As a result of this approach we have five unique tools that can subdivide the spermatogonial population. These will allow us to identify SSC and also subsequent cells in the differentiation process.

In order to generate a faster method of assessing spermatogonial populations we screened α6β1+ cells for the expression of 12 genes including c-kit, pluripotency markers (nanog, oct-4, stella) and genes postulated to mark SSCs (PLZF). We can now identify potential SSC on the basis of high (>3 fold increase in α6β1+ cells) expression of 4 genes and low (>3 fold increase in α6β1- cells) expression of 3 genes as well as the absence of expression of 2 genes that are limited to only α6β1- cells. Immunocytochemical analysis of protein expression revealed different patterns of expression. While we could isolate populations enriched for PLZF gene expression, few cells in the population expressed the protein. In contrast we identified subpopulations with reduced oct4 gene expression where most cells still expressed the protein. These results highlight the importance of posttranscriptional control in spermatogonial differentiation.

In conclusion, we are able to identify subpopulations of spermatogonia and in doing so we have found further evidence of the role of translational control in maintaining stem cell populations.

NUTRIENTS AND INSULIN SIGNALING REGULATE THE GERMLINE STEM CELL DIVISION RATE VIA CENTROSOME ORIENTATION

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A steady supply of differentiated cells from adult stem cells is critical to maintain tissue homeostasis. Defective control of stem cell number either results in loss of stem cells leading to tissue degeneration, or excess proliferation of stem cells leading to tumorigenesis. The *Drosophila* male germline stem cell (GSC) provides an excellent model system to study stem cell behavior in the context of the stem cell niche that is located at the tip of the testis. GSCs attach to a cluster of somatic cells called the hub, and divide asymmetrically by orienting their mitotic spindle perpendicularly to the hub. Stereotypical positioning of centrosomes with respect to the hub sets up this spindle orientation. We have shown that GSCs with misoriented centrosomes, where neither of the two centrosomes is juxtaposed to the hub-GSC junction, undergo cell cycle arrest until their centrosomes reorient, suggesting a novel cell cycle checkpoint to monitor correct centrosome positioning.

Here we show that nutrition, through the insulin receptor-Akt pathway, mediates centrosome orientation in GSCs. Flies grown in poor nutrient conditions or with low insulin signaling have a significantly higher frequency of GSCs with misoriented centrosomes and have mislocalized Apc2 protein, leading to slowed cell cycle progression. This response appears to be mediated via the insulin signaling pathway; expression of constitutively active insulin receptor or ectopic Apc2 expression in GSCs restores centrosome orientation even when flies are cultured in poor nutrient conditions. Mutant GSCs defective in the centrosome orientation checkpoint, such as those that are centrosomin (cnn) mutant or those expressing dominant negative E-cadherin (Inaba et al.) do not undergo cell cycle slow down even in poor nutrient conditions, demonstrating that the centrosome orientation checkpoint is indeed a critical process to regulate the stem cell proliferation rate in response to nutrient availability. We propose that nutrient conditions modulate GSC division rates by controlling centrosome orientation, which can regulate cell cycle progression via the centrosome orientation checkpoint. Our data suggest a mechanism whereby nutrients and insulin signaling function to modulate stem cell proliferation and maintain tissue homeostasis.

REGULATION OF MALE AND FEMALE GAMETOGENESIS BY TWO DISTINCT CPEB PARALOGS IN PLANARIA.

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The planarian *Schmidtea mediterranea* reproduces asexually by transverse fission and sexually by hermaphroditic cross-fertilization. Both modes of reproduction depend on neoblasts, a population of adult pluripotent stem cells, distributed throughout the planarian body. Neoblasts differentiate into soma during the regenerative process of asexual reproduction, and give rise to *nanos*-expressing cells via inductive specification of germ cells. Post-transcriptional regulation by Pumilio, which is required for neoblast maintenance, is predicted to be modulated in the presence of its binding partner Nanos during germ cell specification. We find that another Pumilio-binding protein, the Cytoplasmic Polyadenylation Element Binding protein (CPEB), has two homologs in *S. mediterranea*. *SmedCPEB-1* expression is detected in ovaries, whereas *SmedCPEB-2* is detected in the brain and testes. Disruption of expression of either *CPEB* paralog leads to infertility. *SmedCPEB-2* RNAi results in a block in spermatogenesis, leading to the accumulation of arrested spermatogonia. By contrast, testes of *SmedCPEB-1(RNAi)* animals develop normally, suggesting that their infertility may be due to defects in oogenesis. The separate analysis of ancestral oogenesis-specific and spermatogenesis-specific CPEB paralogs in this simple organism will help us understand the function and diversification of the multiple members of this family of proteins found in higher organisms.

THE ROLE OF THE TRANSCRIPTION FACTOR GCNF IN GERM CELL DEVELOPMENT IN MICE

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The germ cell nuclear factor (GCNF) is a member of the nuclear receptor super family of transcription factors. GCNF expression during gastrulation and neurulation is critical for normal embryogenesis in mice. GCNF represses expression of the POU domain transcription factor Oct4 during mouse post-implantation development in vivo. Oct4 is thus down-regulated during female gonadal development, when the germ cells enter meiosis, which is a process important for reproduction, but one that is rare in germ cells derived from embryonic stem cells in vitro. One aim of our work is to better define the role of GCNF during mouse germ cell development in vivo. We observed a steady decrease in pluripotency-associated gene activity with a concomitant up-regulation of GCNF expression in germ cells derived from developing fetal gonads one day prior to the onset of meiosis. Meiosis-associated genes were then up-regulated at onset of meiosis. These findings suggests that GCNF may repress Oct4 expression in female germ cells and that it plays a role in initiation of meiosis or in activation of meiosis-associated genes in female germ cells. To further investigate the role of GCNF in meiotic processes in male germ cells we generated a GCNF knock-down model to monitor the effect of GCNF during spermatogenesis in a functional manner. The ultimate goal of our studies is to better understand key mechanisms during germ cell development that will serve the in vitro derivation of healthy and functional gametes.

ASYMMETRIC CYTOKINESIS AND MIDBODY INHERITANCE DURING *DROSOPHILA* GERMLINE STEM CELL DIVISION.

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The balance between stem cell self-renewal and differentiation is critical to maintain tissue homeostasis. During steady-state tissue homeostasis, this can be achieved by asymmetric cell division, which is used by many stem cells. Asymmetric stem cell division is achieved by asymmetric segregation of intrinsic fate determinants and/or placement of daughter cells in different microenvironments.

In *Drosophila* testis, germline stem cells (GSCs) normally divide asymmetrically, giving rise to one self-renewing stem cell and one differentiating daughter cell, called gonialblast (GB). This is accomplished by stereotypical positioning of the centrosomes, which sets up the mitotic spindle perpendicular to the hub, the major component of the stem cell niche. We have shown that the mother centrosome is always positioned close to the hub, while the daughter migrates toward the opposite side. It remains unknown whether the mother centrosome and/or daughter centrosomes harbor any fate determining factors that contribute to the asymmetric outcome of the GSC division.

It is known that cytokinesis is asymmetric in some systems such as HeLa cells and mammalian neuroblasts. Toward the end of mitosis, an intermediate structure known as the midbody is formed, consisting of central spindle microtubules and contractile ring derivative (midbody ring (MR)). During the last step of cytokinesis, abscission, the pinching off of the cell membrane occurs normally only from one side of the MR resulting in asymmetric inheritance of the midbody. It has been reported that centrosomal proteins such as Cep55 and centriolin translocate from the centrosome to the midbody during abscission, which is required for trafficking secretory vesicles essential for cytokinesis.

Here we report that the cytotkinesis of *Drosophila* male and female GSCs is asymmetric in that the MR is inherited asymmetrically: In the male GSC divisions the MR is always inherited by the gonialblast, the differentiating daughter cell, whereas in the female GSCs, the MR is consistently inherited by the GSC. We also found that MR remnant is excluded from the male germline cells and degraded in the somatic cells (cyst stem cells or cyst cells), while in the female germline cells the MR remnant is incorporated into the spectrosome, where it is eventually degraded. Interestingly, the MR inheritance pattern was randomized in cnn mutants, an essential component of the pericentriolar material, suggesting that the centrosome plays a key role in the asymmetric cytokinesis. Taken together, our results illuminate another asymmetry during asymmetric stem cell division.

THE POLY(A) POLYMERASE GLD2 IS REQUIRED FOR LATE SPERMATOGENESIS IN *DROSOPHILA MELANOGASTER*.

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The DNA of a developing sperm is not accessible for transcription during much of spermatogenesis in *Drosophila melanogaster*. Most transcripts needed for sperm development are synthesized in pre-meiotic spermatocytes. However, spermatogenesis requires stage-specific production of proteins, and thus necessitates an uncoupling of transcription and translation. In females, developing oocytes utilize poly(A) tail alterations to ensure that dormant transcripts are not prematurely translated: a transcript with a short poly(A) tail will remain untranslated, while elongating the long poly(A) tail permits protein production. An ovary-expressed cytoplasmic poly(A) polymerase called WISP accomplishes such poly(A) extension in the female germline. Here we examine the possibility that the testis-expressed WISP paralog, GLD2, plays a similar role in the male germline. We show that GLD2 deficiency causes male sterility. GLD2-deficient males do not produce mature sperm. Spermatogenesis up to and including meiosis appears normal in the absence of GLD2, but post-meiotic spermatid development rapidly becomes abnormal. Nuclear bundling and F-actin assembly is defective in GLD2-deficient testes, and nuclei fail to undergo chromatin reorganization in elongated spermatids. We find that GLD2 affects translation of protamines as well as stability of dynamin and transition protein transcripts. Our results indicate that GLD2 is an important regulator of late spermatogenesis.

DYNAMICS OF NUCLEAR MEMBRANES AND RNPS IN AGING AND STRESSED OOCYTES

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In many animal species, oocytes arrest in meiosis until they are fertilized. Several cytological studies have reported changes in the cytoplasm of these arrested oocytes, including formation of annulate lamellae and morphological changes in mitochondria. We are focusing on understanding the regulation and function of large ribonucleoprotein (RNP) granules that assemble in the germlines of *Caenorhabditis* nematodes that are either stressed, or in which ovulation is arrested due to old age or an absence of sperm. The large RNP granules are hypothesized to regulate mRNA stability or translation in arrested or stressed oocytes when fertilization is delayed (Jud et al., 2008). To complement an ongoing functional RNAi screen designed to identify genes required for large RNP granules to assemble, we have developed protocols for transmission electron microscopy that reveal novel nuclear membrane structures in close proximity to RNPs in arrested and stressed oocytes. In these oocytes, large nuclear blebs composed of up to four bilayer membranes, form along the nuclear membrane. The blebs appear to detach from the nuclear membrane, as they are also detected in the cytoplasm. To better determine the frequency and distribution of nuclear blebs we have used confocal microscopy and confirmed that while small nuclear blebs are occasionally seen in oocytes of mated females, larger nuclear blebs are observed eight times more often in arrested or stressed oocytes. Germ granules localize in close proximity to the detached nuclear blebs. In more cortical regions of the arrested or stressed oocytes, the ultrastructure of larger RNP granules was examined. The largest RNP granules were heterogenous in electron density and had irregular outlines; numerous mitochondria were often closely associated with the RNP granules, and large stacks of ER were prominent throughout the cytoplasm. Several examples of presumptive annulate lamellae were also seen for the first time in worms; stacked membranes containing nuclear pores that were aligned within neighboring membranes were seen adjacent to, or directly contacting, large RNP granules. Finally, we show these cellular responses to stress and prolonged quiescence are conserved, at least from *C. remanei* to *C. elegans*. We speculate that the appearance of nuclear blebs, their detachment from the nuclear membrane, and the assembly of annulate lamellae are a coordinated cellular response that promotes the formation of large RNP granules in the cortex of stressed or arrested ooctyes.

VRK1 HAS DIFFERENT YET ESSENTIAL ROLES IN MALE AND FEMALE GERM CELL DEVELOPMENT

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The kinase VRK1 (vaccinia-related kinase 1) is conserved in humans, mice, *Xenopus*, *Drosophila*, and *C. elegans* and is known to phosphorylate a variety of proteins involved in regulating the cell cycle, including p53. Previous studies in the Reinke lab have shown that *C. elegans* VRK-1 and the homolog of p53, CEP-1, are enriched in the germline stem cells of *C. elegans*. Additional investigation of these two proteins within the germ line indicates that VRK-1 negatively regulates p53/CEP-1.

In order to further explore the function of VRK1 in a mammalian system, we have generated mice that have a gene trap insertion, rrr178, within *Vrk1*. Mice that are homozygous for this mutated allele (*Vrk1-/-*) are viable and healthy at birth. However, female *Vrk1-/-* mice are sterile from the onset of sexual maturity and male *Vrk1-/-* mice are fertile but then become sterile by 70 d.p.p..

Current work on this project has been focused upon characterizing the *Vrk1-/-* mice and determining the cause of the sterility observed. Male *Vrk1-/-* mice become sterile due to a block in spermatogenesis. Female *Vrk1-/-* mice appear to be sterile due to oocyte defects that manifest during the resumption of meiosis prior to completing prophase I. We have also built a double mutant between *Vrk1* and *p53* in order to investigate the proposed interaction between VRK1 and p53 observed in *C. elegans*. We found that this relationship is not conserved in mouse germ cells.

Ultimately, our data in mice are consistent with previously defined functions of VRK1 in germ cells of other organisms. The proliferation defect during spermatogenesis is reminiscent of the germline stem cell defect observed in *C. elegans*. Additionally, the defect in meiosis I of oogenesis is similar to the meiotic defect described in *Drosophila*. The difference in the phenotypes between the two sexes is likely due to the fact that proliferation occurs in adult spermatocytes but not oocytes. Therefore, in females, the function of VRK1 in meiosis is not masked by its requirement during proliferation.

GENETIC VARIANTS ASSOCIATED WITH FOLLICLE NUMBER IN WOMEN

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Human female fertility and the quantity and quality of oocytes, or ovarian reserve, are highly variable and complex traits with possible genetic and environmental determinants. However, due to the inaccessibility of early human development, the underlying genes and genetic variation have yet to be determined. One measure of ovarian reserve is the antral follicle count (AFC), which is assessed by ultrasound and is an accurate reflection of the number of oocytes remaining within the ovaries. To test the hypothesis that the ovarian reserve is largely determined by multiple genetic factors that impact early germ cell/follicle formation, we conducted a novel study to identify variants associated with AFC and several reproductive hormone profiles. We analyzed 245 Caucasian women aged 25–45, who were assessed for clinical, anthropometric, and lifestyle factors and tested 690,302 SNPs across the genome for association with AFC and hormonal markers. AFC declined with age and was associated with serum anti-Mullerian hormone (AMH) and follicle stimulating hormone (FSH) levels, which play critical roles in follicle development and reproduction. Six SNPs demonstrated at least nominal association with AFC ($P = 7.90 \times 10^{-8}$–2.78×10^{-6}). One SNP at 7q36.1 was significant at the genome-wide level with increases of 6.9 ± 2.0 (mean ± SEM) follicles and 8.2 ± 2.3 follicles for the alternate genotypes. Several SNPs at 6p23, 12p13.1, and 5p13.3, were nominally associated with AMH and FSH ($P = 2.96 \times 10^{-7}$–1.47×10^{-6}). Potentially relevant SNPs localized within or near several genes and genomic regions of high linkage disequilibrium and therefore may be associated with causative alleles or genes. Smoking and previous oral contraceptive use both had marginally significant effects, suggesting additional involvement of environmental influences. Current work is focused at examining genetic and environmental associations with AFC in several other ethnic groups, including African Americans and functional studies on the development of germ cells from induced pluripotent stem cell (iPSC) lines generated from women of this study with extremes of follicle number. This community-based genome-wide study provides the first evidence for genetic variants associated with follicle number and hormonal markers of ovarian reserve in women *(funded by R01HD044876 of NICHD/NIA and UL1RR024131 of NIH/NCRR)*.

THE *C. ELEGANS* GERMINAL CENTER KINASE GCK-1 IS A P-GRANULE COMPONENT REQUIRED FOR P-GRANULE INTEGRITY IN PACHYTENE AND LATER STAGES OF OOGENESIS

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Germ cells of many species harbor specific RNPs that are thought to regulate RNA metabolism and the translation of proteins necessary for the maintenance and development of the germ line. In *C. elegans*, these RNPs, P-granules, are segregated to the germ lineage in the first embryonic mitotic division and are associated with germ nuclei throughout most of germ line development. Although many RNAs and proteins are associated with P-granules, their function and mechanistic relationship remains enigmatic. We discovered that *C. elegans* GCK-1, a member the Ste-20-Germinal Center Kinase family, co-localizes with P-granules, most prominently in mid- to late pachytene germ cells.

Depletion of GCK-1 results in the abnormal progression meiotic prophase I of oogenesis, a process that is regulated by the ERK MAP kinase MPK-1. Loss of GCK-1 results in hyper-activation of MPK-1 during pachytene, suggesting that GCK-1 functions in germ cell development as a negative regulator of the MAP kinase pathway. Interestingly, in GCK-1-depleted gonads P-granule-specific antibodies revealed that while P-granule immunostaining is present in mitotic and early meiotic germ cells, it is greatly reduced once cells progress into pachytene and is largely absent from the small, abnormal oocytes that populate the proximal end of GCK-1-deficient gonads. These results suggest that GCK-1 is a P-granule component and is required for their integrity at a distinct stage of germ cell development.

GCK-1 is a binding partner of the Aurora A kinase AIR-1. AIR-1 interacts with and phosphorylates the GCK-1 N-terminus in a region that overlaps a MPK-1 binding domain. AIR-1 phosphorylation was mapped to a single serine that lies in the GCK-1 kinase domain just upstream of the MPK-1 docking site. To determine whether AIR-1 phosphorylates GCK-1 at this site in vivo (S44), we raised a phospho-specific antibody (pGCK-1(S44)). Immunostaining of wt and GCK-1-depleted gonads revealed that p-GCK-1(S44) specifically decorates mid- to late pachytene stage P-granules in a pattern very similar to that revealed by an antibody against recombinant GCK-1. pGCK-1(S44) immunostaining is greatly reduced in the germ line of hermaphrodites homozygous for an air-1 hypomorphic allele. The data above suggest a model whereby AIR-1 phosphorylates GCK-1 during pachytene, leading to the recruitment of GCK-1 to perinuclear P-granules, which is required for appropriate levels of MPK-1 activation and P-granule stability. Experiments to address the role of GCK-1 S44 phosphorylation in P-granule functionality and germ line development are underway.

GDNF OVEREXPRESSION BLOCKS DIFFERENTIATION OF SPERMATOGONIAL STEM CELLS AND ALTERS THEIR INTERACTION WITH THE EXTRACELLULAR MATRIX

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Stem cell self-renewal and differentiation are tightly controlled in a well-defined location within a tissue, called the niche. Within the testis, the niche that supports spermatogonial stem cells includes the somatic Sertoli cells, the extracellular matrix on which they lie, and probably soluble factors within the interstitium. Glial cell line derived neurotrophic factor (GDNF) is expressed in Sertoli cells and the loss of this factor leads to an age-dependent depletion of the SSCs (Meng X et al., 2000). GDNF binds to the GFRSpecial character-1 receptor, which is linked to the plasma membrane by a glycosylphasphatidylinositol (GPI) anchor, and forms a complex with receptor tyrosine kinase (RET). In this study, we show that expression of *Gdnf* in Sertoli cells is stage-specific, with high levels observed during the early and late stages, the stages when A_{single}, A_{paired} and $A_{aligned}$ spermatogonia proliferate. In contrast, low levels are observed in stages when $A_{aligned}$ spermatogonia differentiate into A1 cells. To investigate the importance of stage-specific expression of GDNF and its effect on germ stem cell proliferation, we generated transgenic mice with a reciprocal expression pattern of *Gdnf* in Sertoli cells using the stage-specific Cathespin-L (*Ctsl*) promoter. Mice with altered expression of GDNF accumulate clusters of PLZF positive-cells. Marker analysis (GFRα-1, SOHLH1 and LIN28) and germ cell transplantation experiments show that cells in the clusters are spermatogonial stem cells. BrdU labeling demonstrated that the rate of proliferation in the clusters is similar to PLZF-positive cells in wild type testes suggesting that GDNF functions in stem cell self-renewal by inhibiting differentiation. BrdU incorpation into PLZF-positive cells after busulfan treatment is also reduced in the transgenic testes suggesting that niche occupancy by PLZF positive cells suppresses proliferation of SSCs. Interestingly, the basement membrane components Laminin A1 and collagen IV are altered near the proliferating clusters of stem cells suggesting that spermatogonial stem cells regulate the extracellular matrix in which they reside.

Reference: Meng X, et al., Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Sci 2000; 1489-1493.

HORMONE-DEPENDENT GERMLINE STEM CELL DIFFERENTIATION IN *DROSOPHILA* IS FINE-TUNED BY MIRNAS

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We performed a candidate-based genetic screen to identify new genes required for germline stem cell maintenance and division. We used an available FRT lethal collection to induce mutations specifically in soma or germline using FLP/FRT mediated recombination. From so far screened 206 pre-selected lines, 53 mutants were chosen for the secondary analysis. Identified genes include transcription factors, signaling transduction molecules, cell cycle regulators and components of the RNAi machinery. Interestingly, some of the found components have been shown to interact with each other. Now our findings show that the hormonal signaling and miRNAs direct germline stem cell differentiation. This effect of hormones is non-cell autonomous, the pathway is activated in soma: the stem cell niche and escort cells, but controls germline behavior. In addition, the hormonal signaling is reassured via the miRNA pathway. Not only is the miRNA expression itself controlled by hormones, miRNAs act in the feedback loops to regulate the strength of the hormonal signaling. This provides the means to fine-tune the signals managing stem cell division, maintenance and differentiation in response to ever-changing extracellular conditions.

DIRECT LINK BETWEEN EPIGENETIC MODIFICATION AND STEM CELL DIFFERENTIATION IN TESTES

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It is well known that epigenetic regulation of gene expression plays crucial roles for maintenance and differentiation of pluripotent stem cells. However, epigenetic characteristics of tissue-specific stem cells and progenitor cells are not well known. The male germ cells have unique feature that they connect each other after cell division, and this system enables us to track spermatogonia differentiation simply by counting chain number. To take this advantage, we analyzed changing of epigenetic modifications during mitotic phase of spermatogenesis in mice to observe whether stem cells can be distinguished from progenitor cells in testes.
First, we found that de novo DNA methyltransferases, Dnmt3a2 and Dnmt3b, did not express in spermatogonial stem cells from As to Aal4, and genomic methylation level of those population was low. At the transition from spermatogonial stem cells to progenitor cells, we found that the expression of de novo DNA methyltransferases were drastically increased and the genomic DNA methylation level became high. In addition, the level of H3K9me2 modification was increased at the same stage with the increase of GLP expression.
Second, we found that global DNA methylation is crucial for differentiation from stem cells to progenitor cells, but not maintenance of stem cells. The differentiation of spermatogonia is severely impaired in Uhrf1 deficient mice. On the contrary, premature expression of Dnmt3b converted stem cells to c-Kit-positive progenitor cells.
These data suggest that the stem cells in testes can be clearly distinguished from the progenitor cells by the difference of epigenetic modifications and higher order chromatin architecture might play crucial roles for transition from the stem cells to the progenitor cells in mice testes.

CTCF DEPENDENT CHROMATIN BIAS CONSTITUTES TRANSIENT EPIGENETIC MEMORY OF THE MOTHER AT THE H19-IGF2 IMPRINTING CONTROL REGION IN PROSPERMATOGONIA

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Genomic imprints --parental allele-specific DNA methylation marks at the differentially methylated regions (DMRs) of imprinted genes-- are erased and reestablished in germ cells according to the individual's sex. Imprint establishment of the paternally methylated germ line DMRs occurs in fetal male germ cells. In prospermatogonia, the two unmethylated alleles exhibit different rates of de novo methylation at the H19/Igf2 imprinting control region (ICR) depending on parental origin.

We investigated the nature of this epigenetic memory using bisulfite sequencing and allele-specific ChIP-SNuPE assays. We found that the chromatin composition in fetal germ cells was biased at the ICR between the two alleles with the maternally inherited allele showing more H3K4me2 and less H3K9me3 than the paternally inherited allele. The chromatin bias and also the delayed methylation establishment in the maternal allele depended on functional CTCF insulator binding sites in the ICR.

Our data suggest that in primordial germ cells, maternally inherited allele-specific CTCF binding sets up allele-specific chromatin differences at the ICR and the erasure of these allele-specific chromatin marks is not complete before the process of the de novo methylation imprint establishment begins. CTCF-dependent allele-specific chromatin composition imposes an allele-specific delay on de novo methylation imprint establishment at the H19/Igf2 ICR in prospermatogonia.

VARIATION AND REPRODUCTIVE SUCCESS: STEM CELL FATE PREDETERMINATION IN DROSOPHILA POLE CELLS

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We are interested to understand how cell to cell variability and qualitative and quantitative differences between cells may contribute to reproductive success. We specifically want to elucidate whether cells are predetermined to acquire stemness – become stem cells. We use *Drosophila* germline stem cell (GSCs) development as a model. GSCs arise from a group of pole cells formed early in embryonic development. Some of the initial parameters that differ between individual pole cells of the same organisms are the amount of inherited germ plasm and the onset of transcription. We mark and follow single pole cells with different parameters throughout the development in order to determine how initial differences between pole cells lead to stem cell fate or differentiation. Elucidating the molecular mechanisms that promote stem cell formation versus differentiation will provide new clues in acquisition and likelihood to acquire stemness in general, which may provide new insight into the mechanisms that operate *in vivo* to select stem cells both in their normal environment and also in disease states such as cancer. Understanding the molecular mechanisms of stem cell selection will allow us to manipulate their state and may provide new approaches to regenerative medicine.

CHARACTERIZATION OF DAZL-PABP INTERACTIONS IN MAMMALIAN GAMETOGENESIS

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Gametogenesis is a highly complex process regulated by stringent mechanisms which control both temporal and spatial gene expression. Translational regulation is essential for the correct progression of gametogenesis. Deleted in Azoospermia-like (Dazl) is an RNA-binding protein that regulates the translation of specific mRNAs in vertebrate germ cells. A model for the function of Dazl in stimulating translation initiation has been proposed based on its interaction with poly(A)-binding proteins (PABPs), which are basal translation initiation factors.
As the first step towards exploring the physiological relevance of PABP-Dazl interaction in mammalian gametogenesis I am characterizing the expression pattern of the different PABP family members to establish the extent to which they co-localise with Dazl within gonads. Immunofluorescence shows that in adult mouse testes PABP1 expression is first detectable in the cytoplasm of spermatocytes, peaks in the cytoplasm of round spermatids, starts to decrease in elongating spermatids, and is absent in mature spermatozoa. With the exception of spermatozoa, PABP4 is expressed throughout spermatogenesis; with a peak of expression in the cytoplasm of spermatogonia and spermatocytes. As Dazl expression peaks in the cytoplasm of spermatogonia and spermatocytes, these results indicate that there is a transitory co-localization of Dazl and PABP1 in spermatocytes, and Dazl and PABP4 in spermatogonia and spermatocytes. In order to assess whether mammalian PABP family members interact with murine Dazl, a yeast two-hybrid approach was taken. This showed that mouse Dazl interacts with PABP1, similar to observations in Xenopus, suggesting that this interaction is conserved. Interestingly, we found that testes express four different splice-forms of *PABP4*. However none of these appear to interact with Dazl, providing the first example of a PABP specific protein-protein interaction. These results lead us to suggest that during mammalian spermatogenesis, Dazl's role in stimulating the translation of specific mRNAs may be primarily mediated by an interaction with PABP1.

SEXUAL COMPATIBILITY BETWEEN THE GERMLINE AND SOMA OF THE *DROSOPHILA* TESTIS

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Phenotypic differences between the sexes are often most apparent in the somatic cells of an organism. However, in many animals, germ cells have independent means of determining their own sexual identity. In such cases, sex reversal of the soma causes germline loss, likely due to an incompatibility between germ cells and somatic cells of opposing sexual identities. *Drosophila* germline and soma use separate pathways to establish sexual identity but communication between the soma and germline can also influence a germ cell's sex-specific gene expression. Somatic influence may make germline and somatic sex similar enough for development to proceed normally in some respects, while the intrinsic sexual identity may prevent proper development in other respects.

Mutations in the female somatic sexual identity pathway (tra or tra2 mutations) give the somatic cells of an XX fly male identity. These flies form normal-looking gonads in embryogenesis, implying that later aspects of gonad development are impaired. I examined germline stem cell (GSC)-niche interactions and differentiation in the testis of XX flies with sexually transformed soma to determine the nature of this germline-soma incompatibility. I find that XX germline retains female character in a male soma. I find that physical interactions between XX GSCs and the hub (a component of the male stem cell niche) are mostly normal while GSC-niche signaling is perturbed. Specifically, a transcription factor (Stat92E) that is highly upregulated in XY GSCs in response to hub signals is not as highly upregulated in XX GSCs. Expansion of the hub in XX males also implies that GSC-niche communication is abnormal. A failure for XX germ cells and male soma to properly communicate could explain the differentiation defects observed in pseudomale germline. Male somatic cells appear to only weakly associate with XX differentiating cysts. Additionally, many cysts undergo apoptotic death, likely due to aberrant signaling within the cyst.

Overall, germline and soma sexual compatibility is important for GSC-niche signaling and cyst differentiation. Interestingly, XY pseudofemales also lose germline and XY germ cells retain male character in a female soma. Future analysis will reveal whether other aspects of pseudofemale germline-soma interactions have defects similar to those of pseudomales. Such studies contribute to our understanding of many animal systems where sex reversal or aneuploidy results in germline defects, including mice and humans, and highlight the necessity of matching germline-soma sexual identities throughout gametogenesis.

TRANSLATIONAL REGULATION AND THE CONTROL OF MEIOTIC MATURATION IN *C. ELEGANS*.

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C. elegans oocytes undergo meiotic maturation and ovulation in response to major sperm proteins (MSPs), hormonal signals released from sperm. MSPs also function as cytoskeletal elements in the actin-independent motility of nematode sperm. The follicle-like gonadal sheath cells, which form gap junctions with oocytes and contract to drive ovulation, function as the initial MSP sensors. G protein-stimulated cAMP-dependent protein kinase A activity in the gonadal sheath cells is required for all germline responses to MSPs (abstract by S. Kim, this meeting). In the germ line, the meiotic maturation response requires the combined activities of two Tis11-like CCCH zinc-finger proteins, OMA-1 and OMA-2 [Detwiler et al. (2001) Dev. Cell 1: 187]. To elucidate the mechanisms by which the OMA proteins promote meiotic maturation, we developed methods for purifying OMA ribonucleoprotein particles (OMA RNPs) from oocytes. OMA RNPs were purified in both the presence and absence of MSP signaling and proteins were identified using mass spectrometry. OMA RNPs contain a large number of germline-expressed RNA-binding proteins including translational activators and repressors. To distinguish core OMA RNP components from those tethered by RNA, we purified OMA complexes after treatment with RNase A. Core OMA RNP components include multiple subunits of the GLD-2 poly(A) polymerase and CCR4/NOT1 deadenylase complexes and many RNA-binding proteins, including, MEX-3, MEX-1, and LIN-41. OMA RNP components that appear to be tethered primarily via RNA associations include the P-body proteins CGH-1/p54 and CAR-1/Rap55. No major differences were observed in the composition of OMA RNPs in the presence and absence of MSP.

OMA RNPs are cytoplasmically dispersed in oocytes when MSP is present but are found in stress granule-like foci when MSP is absent, as has been observed for a number of germline-expressed RNA-binding proteins [Schisa et al. (2001) Development 128: 1287; Jud et al. (2008) Dev. Biol 318: 38]. These data are consistent with the hypothesis that OMA-1/2 might regulate the translation of mRNAs encoding proteins involved in meiotic resumption or the oocyte-to-embryo transition. To test the role of OMA RNP components in translational regulation, we used microarrays to define mRNA components of OMA RNPs. This analysis identified a set of approximately 400 germline-expressed mRNAs that are enriched in OMA RNPs. Mirroring the results of the proteomic analyses, the mRNA composition of OMA RNPs was not dependent on the presence of the MSP signal. We will investigate whether specific mRNAs are translationally regulated by OMA-associated proteins, potentially in response to the MSP signal.

HBP1 AND THE REGULATION OF MITOTIC ARREST IN FETAL MALE GERM CELLS.

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During development somatic cell cues direct sex-specific differentiation of germ cells that is characterised by distinct cell cycle states. At 12.5 days post coitum in a testis, XY germ cells stop proliferating and enter G1/G0 arrest. Whilst it is hypothesised that errors in cell cycle control during development precede the formation of testicular germ cell tumours, the mechanism of cell cycle control at this time has not been thoroughly investigated. The high mobility group box transcription factor 1 (HBP1) suppresses proliferation and promotes differentiation in various cell types and was recently identified within the XY germ cells at the appropriate time of sex differentiation. Therefore we hypothesise that HBP1 is involved in initiating and/or maintaining XY germ cell G1/G0 arrest. In order to test this hypothesis we firstly characterised Hbp1 expression during germ cell development and identified two splice variants that display different sub-cellular localisations within the XY germ cells. Next, Hbp1-LacZ reporter lines were generated to aid in understanding the germ cell-specific regulation of these transcripts. Lastly, we used a loss-of-function approach by generating and analysing the genetrap mutation for Hbp1.

THE *DROSOPHILA* RECEPTOR TYROSINE PHOSPHATASE LAR REGULATES CELL ADHESION BETWEEN MALE GERMLINE STEM CELLS AND THE HUB.

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Adult stem cells are undifferentiated reserves that maintain tissue homeostasis by their ability to self-renew and to give rise to differentiated cells. The decision between the alternate fates of stem cells need to be tightly regulated, misregulation can result in tissue degeneration or in development of cancers. *In vivo* studies show that adult stem cells reside in and are supported by a specialized microenvironment called a niche. The niche maintains stem cells by providing signals that promote self-renewal and spatial cues for oriented cell divisions. Cell adhesion to the niche is key to ensure that stem cells remain in close proximity to the niche. We examined the role of cell adhesion in maintaining stem cell fate using the *Drosophila* male germline as a model system. We hypothesize that one outcome of the niche signal important for stem cell maintenance is to regulate the cell adhesion between stem cells and the niche. We have identified a role for regulators of protein tyrosine phosphorylation in attachment to and maintenance of *Drosophila* male germ line stem cells (**GSCs**) in the hub, a component of the niche microenvironment in this system. The receptor tyrosine phosphatase, Leukocyte Antigen Receptor (**Lar**) is expressed in GSCs and early transit-amplifying cells, while the ligand of Lar, Dally-like, is expressed in the hub. Loss of *lar* function in the germline results in fewer GSCs due to weakened adherens junctions between the hub and GSC as observed by ultrastructural analysis. Strikingly, overexpression of the tyrosine kinase Abl in GSCs results in stem cell loss too. Lar and Abl are functional antagonist and have been shown to regulate the function of the F-actin regulator, Ena in *Drosophila* nervous system. Ena is expressed in GSCs and localizes to the hub-GSC interface and loss of *ena* function results in GSC loss suggesting that Lar and Abl regulate adhesion between the hub and GSC through Ena. Interestingly, Ena is a target of STAT transcription factor that is activated in response to the signal from the hub suggesting that multiple signaling pathways converge to ensure maintenance of attachment of GSCs to the hub. Our current model based on these data is that Lar through its interaction with Dally-like activates and/or localizes Ena to the hub-GSC interface to promote hub-GSC adhesion by localized regulation of cortical F-actin assembly.

A METHOD FOR PURIFYING NUCLEI FROM DIFFERENT TISSUES IN C. ELEGANS

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Obtaining tissue- or cell-type specific expression profile and chromatin profiles is one of the challenges in creating complete pictures of the genome landscapes. Our group recently published a simple method for the cell-type specific purification of nuclei from Arabidopsis roots1. The method, termed INTACT (Isolation of Nuclei TAgged in specific Cell Types), relies on a nuclear envelope tag that is biotinylated in vivo, which allows for affinity purification of the biotin-tagged nuclei.
We have adapted the INTACT method to C. elegans tissues. In a first proof of principle strain, an outer nuclear pore complex protein is fused to mCherry for visualization and a BLRP tag (Biotin Ligase Recognition Peptide), and its expression is driven by the germline-specific pie-1 promoter. The E. coli biotin ligase BirA is expressed ubiquitously, mediating in vivo biotinylation. Using this approach, germline nuclei can be efficiently purified. Profiling of the affinity purified nuclei is ongoing. The system will allow purification of nuclei from different tissues at different developmental stages from embryos to adults.

1 A Simple Method for Gene Expression and Chromatin Profiling of Individual Cell Types within a Tissue. Deal RB, Henikoff S. Dev Cell. 2010 18(6) 1030-40

HIGH RESOLUTION TRANSCRIPTOME AND PROTEOME PROFILING DURING OOCYTE TO EMBRYO TRANSITION IN *CAENORHABDITIS ELEGANS*

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The oocyte-to-embryo transition is a fundamental event in early development and one of the most dynamic processes in biology. During this transition the fusion of two highly specialized cells, sperm and oocyte, triggers the development of a mitotically-dividing totipotent embryo. In *C.elegans*, within 40 minutes after fertilization, the embryo has completed oocyte meiotic maturation, pronuclear fusion and the first intrinsically asymmetric division is initiated. This includes organelle and cytoskeletal remodeling, protein synthesis and RNA and protein degradation. This entire developmental process is thought to be driven by post-transcriptional gene regulation. Although a number of key players are known, the molecular underpinnings of this transition remain poorly understood. Therefore, we sought to obtain a global characterization of changes on coding and non-coding transcriptome and proteome during this critical developmental progression in the nematode *C. elegans*. To this end, we developed a new method which uses in vivo SILAC to quantify changes in the abundance of thousands of proteins between two *C. elegans* samples (Thierfelder *et al*, unpublished). We combined this method with our recently developed method to obtain synchronized embryonic samples ("eFACS", Stoeckius *et al*, 2009). We combined these techniques to measure mRNA, small RNAs (microRNAs, 21URNAs, 22G-RNAs, 26G-RNAs, and others) and the amount of thousands of proteins in oocytes, 1-cell-, 2-4 cell-stage embryos and sperm. We discern highly orchestrated changes on all levels (mRNAs, proteins, small RNAs). We also observe evidence for sperm derived RNAs and proteins in the zygote, suggesting an unexplored substantial paternal contribution to early development. We will report on the results of the currently ongoing analyses and validations of our data sets.

THE TRANSLATIONAL REGULATOR PUF-8 PROMOTES MRNA PROCESSING/EXPORT IN C. ELEGANS GERM CELL NUCLEUS

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Members of PUF family are highly conserved RNA-binding proteins that control processes as diverse as germline stem cell maintenance, embryonic patterning, and neuronal function and differentiation. Based on the examples of fly Pumilio and worm FBF, PUF proteins have been proposed to function as translational regulators by binding to specific 3'UTR sequences. We find that the Caenorhabditis elegans PUF protein PUF-8 is present in the nucleus as well, where it promotes mRNA processing/export. We provide genetic evidence that PUF-8 functions redundantly with several components of the splicing and mRNA export machineries during germ cell development. It co-localizes at the nuclear periphery of germ cells with TCER-1, whose yeast and mammalian orthologs link transcription and splicing. Both proteins are redundantly essential for the formation of spliced versions of several germline mRNAs. Finally, both PUF-8 and TCER-1 physically interact with proteins involved in splicing and export of mRNAs. In addition, simultaneous depletion of PUF-8 and any one of several proteins involved in either processing or export of mRNAs affect the integrity of germ granules, which suggest a role for mRNAs transiting through the germ granule for its integrity. Our results reveal that the PUF proteins – long thought to function as sequence-specific translational regulators – may have a wider role in mRNA metabolism, both in the nucleus as well as cytoplasm, and influence the expression of a much larger set of genes than what is currently known.

DIFFERENTIATION OF STEM/PROGENITOR SPERMATOGONIA IS CONTROLLED BY GERM CELLS OF ADVANCED STAGES IN MOUSE SPERMATOGENESIS

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The maintenance of animal organs depends on a proper timing of the stem cell differentiation. Seminiferous tubules are the spermatogenic center of mammalian testes. In the tubules, undifferentiated spermatogonia involving stem cells periodically produces differentiating germ cells committed to sperms with cyclical supports by Sertoli cells, which are sole somatic cells inside the tubule. Our study shows that retinoic acid (RA) signaling simultaneously induces spermatogonial differentiation and resets the cyclic gene expression of Sertoli cells, which results in a coordination between germ cells and Sertoli cells. In seminiferous tubules, the RA metabolism is controlled locally by coordinated expression of up-take receptor and metabolic enzymes that are expressed separately in Sertoli cells and germ cells of various stages. In our study, feedback regulation of such genes by RA was observed in Sertoli cells, but not in germ cells. Our result suggests that germ cells dominantly control local RA metabolism involving Sertoli cells in seminiferous tubules. Given the strong expression of RA synthesis gene beginning in a particular differentiating step of germ cells (spermatocytes), we propose that differentiating germ cells cue for the differentiation of undifferentiated spermatogonia through RA signaling and the periodical emergence of the particular differentiating germ cells ensures the proper timing of periodical differentiation of stem cells. In other systems, differentiating cells might control the stem cell differentiation as well.

INTERACTION BETWEEN NANOS2 AND CCR4-NOT DEADENYLATION COMPLEX IS ESSENTIAL FOR MALE GERM CELL DEVELOPMENT IN MOUSE.

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In the mouse, the primordial germ cells (PGCs) are segregated from the somatic cell lineage at an early gastrulation stage. Once generated, PGCs continue to proliferate and migrate through somatic tissues to reach the gonads. Although the PGCs have potency to produce both oogonia and spermatogonia at this migrating stage, sexual differentiation is induced after their colonization with the somatic cells in the embryonic gonads. The PGCs that colonize the female gonads (female gonocytes) enter meiosis and proceed to the diplotene stage of meiotic prophase-I, whereas PGCs colonizing the male gonads (male gonocytes) become arrested at G1/G0 and undergo genome-wide DNA methylation and paternal imprinting.
Nanos is an evolutionarily conserved protein implicated in germ cell development in many species. Three Nanos homologs, NANOS1–3, exist in the mouse, among which NANOS2 expression begins only in male gonocytes and a loss of this protein results in a complete lack of male germ cells. We have previously reported that NANOS2 plays a key role during the sexual development of germ cells by suppressing meiosis and promoting male-type differentiation in the male gonocytes. A molecular mechanism underlying how NANOS2 suppresses meiosis is suggested to be post-transcriptional suppression of meiotic genes. Since NANOS2 has also been shown to interact with CCR4-NOT deadenylation complex and some mRNAs of meiotic genes, NANOS2 is likely to recruit CCR4-NOT deadenylation complex to the meiotic mRNAs for their degradation, and thus prevent male gonocytes from abnormal entry to meiosis. However, it is still not clear whether such pleiotropic functions of NANOS2 could be explained only through this interaction.
In our present study, we explore the physiological significance of the interaction between NANOS2 and CCR4-NOT deadenylation complex in male gonocytes. We first found that NANOS2 directly bound to the C-terminal region of CNOT1, a scaffold protein of CCR4-NOT complex, and identified peptide sequence of NANOS2 essential for this interaction. We next generated a transgenic mouse line that expressed NANOS2 deletion mutant of the peptide sequence under the direct control of Nanos2 enhancer and introduced the transgene into Nanos2-null mice. Then, we observed that the mice showed same phenotypes as those of Nanos2-null mice, indicating that the interaction with CCR4-NOT deadenylation complex is essential for almost all the aspects of NANOS2 function.

PROSTAGLANDINS REGULATE ACTIN DYNAMICS DURING *DROSOPHILA* FOLLICLE DEVELOPMENT

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Dynamic cytoskeletal remodeling events are necessary for many developmental processes, including *Drosophila* follicle development. A *Drosophila* follicle is composed of 16 germline-derived cells, 15 nurse or support cells and one oocyte, that are surrounded by a somatic epithelium. At mid-oogenesis, the nurse cells dramatically reorganize their actin cytoskeleton, strengthening their sub-cortical actin and generating cytoplasmic filaments. These actin structures are necessary for nurse cell dumping, the contractile process during which nurse cells squeeze their cytoplasmic contents into the oocyte providing it with the materials needed for embryonic development. What are the signals that temporally and spatially regulate these actin remodeling events? Previously we have shown that reduced prostaglandin signaling by either pharmacologic inhibition or loss of function mutations in the gene encoding for the *Drosophila* COX-like enzyme, pxt, blocks nurse cell dumping via a loss in the necessary actin structures. We will present evidence supporting the model that prostaglandins, locally acting lipid signals, function as developmental hormones to regulate the cytoskeletal changes during nurse cell dumping. Specifically, PGE_2 acts to prevent, while $PGF_{2\alpha}$ initiates actin remodeling. Using a combined approach of pharmacology and genetics, we will probe the opposing functions of these two prostaglandins during the actin remodeling necessary for nurse cell dumping.

EXPLORING THE ROLE OF EGF, SCF & GDNF DURING PROLIFERATION AND DIFFERENTIATION OF HUMAN TESTICULAR GERM CELLS *IN VITRO*

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Role of intratesticular factors during various stages of spermatogenesis has been poorly studied due to paucity of human tissue for research and non-availability of an appropriate *in vitro* system. Present study is a step towards gaining insight into the role of growth factors in human testis by developing a suitable organotypic culture system and examining the effect of such factors on expression of stage-specific markers during spermatogenesis.

Normal human testicular tissue obtained from men undergoing orchidectomy for prostate cancer, was cultured as explants and treated with Epidermal Growth Factor (EGF), Stem Cell Factor (SCF) and Glial Cell Derived Neurotrophic Factor (GDNF). The effects of treatment on expression of early and late markers of germ cells (α6Integrin, c-KIT-spermatogonia; Boule, Scp3-spermatocye & TH2B, TP1-spermatid) and somatic cells (StAR-Leydig; SCF-Sertoli cells) were analyzed by Real Time PCR. Immunolocalization of these factors and/ or their receptors in the human testis was also explored. EGF stimulated high expression of all markers except α6 Integrin and localized on both somatic and germ cells, whilst its receptor predominantly localized on the somatic cells. The observed upregulation of somatic markers and the localization of EGFR on somatic cells imply that EGF may initially act on testicular somatic cells and secondarily influence germ cell proliferation and differentiation. SCF induced upregulation of spermatogonial & spermatid markers and transient upregulation of early meiotic marker BOULE. Increased expression of StAR was noted. c-KIT, the receptor for SCF, was found to localize on spermatogonia, early spermatids and Leydig cells. Hence, in addition to its direct effect on proliferation of spermatogonia and maturation of spermatids, SCF may also regulate spermatogenesis indirectly through its effect on Leydig cells. GDNF up-regulated spermatogonial markers as expected, but interestingly also induced upregulation of Boule, Scp3, TH2B, TP1 and SCF. Hence it is tempting to hypothesize, that in addition to its known role in spermatogonial renewal and proliferation, GDNF may also regulate germ cell differentiation via the somatic cells.

Present study demonstrates that human testicular tissue is responsive to EGF, SCF & GDNF, which act as specific autocrine-paracrine mediators in the regulation of proliferation and differentiation of germ cells for successful spermatogenesis, for the first time. Results may be useful while addressing issues like oncofertility, pluripotent stem cell cultivation and infertility.

P GRANULES EXTEND THE NUCLEAR PORE ENVIRONMENT IN THE *C. ELEGANS* GERM LINE

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Ribonucleoprotein (RNP) aggregates called germ granules have been observed in the germ plasm of many species. While depletion of germ granules results in infertility, the role of visible granules within germ plasm has yet to be determined. It was previously thought that in *Drosophila* and *C. elegans* maternally provided germ granules supply the nascent germ line with maternal transcripts necessary for germline formation. More recently, live imaging techniques have exposed the discontinuity of visible germ granules from *Drosophila* nurse cells to the embryonic polar plasm (Snee & Macdonald, 2004), and have shown that P granules in *C. elegans* embryos dissolve and recondense (Brangwynne, et al., 2009). Using *C. elegans* P granules as a model, we are investigating the properties of germ granules and the nucleation potential of individual granule components. A genome-wide RNAi screen revealed the importance of nuclear pores in P granule integrity: depletion of numerous nuclear pore proteins (Nups) results in the detachment of P granules from the periphery of germ cell nuclei (Updike & Strome, 2009). Notably, the *C. elegans* homologs of Vasa, GLH-1, GLH-2, and GLH-4, contain Nup-like FG (phenylalanine-glycine) repeats. Within nuclear pores, weak hydrophobic interactions between the FG repeats of Nups establish a size exclusion barrier between the cytoplasm and nucleus, while also facilitating nuclear transport. We demonstrated that P granules establish a size exclusion barrier similar to that of nuclear pores and, like FG-rich Nups, are held together by weak hydrophobic interactions. To examine the properties of P granule proteins in the absence of other germline factors, we ectopically expressed individual components in the *C. elegans* intestine. We found that neither GLH-1 nor its FG repeat domain can self-aggregate. By multimerizing the FG repeat domain within GLH-1, a localized FG repeat concentration threshold is achieved and perinuclear granules form, suggesting that GLH-1 granule formation requires nucleating components. We show that one of these nucleation factors is the P granule component PGL-1, which self-assembles into small cytoplasmic aggregates and can recruit otherwise diffuse GLH-1 into granules. Our analysis of which components of P granules are necessary for granule assembly and perinuclear location in the germ line and which components are sufficient in the intestine are providing insights into how germ granules form and what functions they may serve. We hypothesize that P granules provide a unique cytoplasmic extension of the nuclear pore environment for post-transcriptional regulation in the germ line.

THREE-DIMENSIONAL GONAD RECONSTRUCTIONS SUPPORT MATURATION OF IN VITRO DERIVED PRIMORDIAL GERM CELLS.

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Primordial germ cells (PGCs) are the founder cells of the entire germ line and their proper development is critical for reproductive fitness. However, the molecular mechanisms that govern PGC formation are poorly understood. Therefore, we developed a model using mouse embryonic stem cells (ESCs) to generate *in vitro* derived primordial germ cells (iPGCs). Using *Oct4-gfp* ESCs, we identified the emergence of a GFP+ population with differentiation. We correlated expression of Oct4-gfp with SSEA1 and cKIT, two cell surface antigens robustly expressed by pre-gonadal *in vivo* PGCs. We used FACS to fractionate SSEA1+/cKIT+ cells and determined that SSEA1+/cKIT^{bright} cells were transcriptionally enriched for migratory PGC genes, indicating that this population corresponds to an immature iPGC population. Gene expression analysis by microarray determined that iPGCs are also transcriptionally distinct from undifferentiated ESCs. Interestingly, iPGCs transcribe mRNA for *Mvh*, a marker of colonization-stage PGCs. However, at the protein level iPGCs are negative for Mvh, indicating that the RNA transcript is not yet translated. Epigenetically, iPGCs are enriched for the histone modification H3K27me3, a marker of E8.5 *in vivo* migratory PGCs. Bisulfite sequencing revealed that iPGCs have initiated demethylation specifically at imprinted loci and not at other methylated regions of the genome. Functionally, iPGCs cultured on feeders in the presence of retinoic acid generated alkaline phosphatase positive self-renewing colonies, confirming their germ cell identity. Together, these results suggest that our iPGC derivation method generates a poised pre-gonadal stage PGC corresponding to approximately E9.5 of development *in vivo*. We hypothesize that iPGCs are capable of undergoing further maturation if placed in the proper niche. To test this, we built a three-dimensional gonad reconstruction model where iPGCs are co-cultured with fetal gonadal somatic cells. H&E staining of reconstructed gonads indicate that gonadal somatic cells organize themselves into distinct structures upon re-aggregation. iPGCs undergo further differentiation to Mvh+ PGCs upon interaction with gonadal somatic cells, while downregulating markers of early PGC development such as Oct4 and SSEA1. These results highlight the importance of the gonad environment for PGC maturation and provide a model to characterize molecular regulation and niche interactions that promote germ cell establishment *in vitro*. Furthermore, this model provides a potential system to generate functional germ cells that could be used as the foundation towards creating novel methods to overcome infertility.

THE *C. ELEGANS* HOMOLOG OF NUCLEOPORIN NUP98 IS REQUIRED FOR P GRANULE INTEGRITY AND POST-TRANSCRIPTIONAL SILENCING IN GERMLINE STEM CELLS

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Germ granules in *C. elegans* (P granules) are present throughout germline development and associate with nuclear pore clusters on germ cell nuclei (Strome and Wood, 1982; Pitt et al., 2000). We recently reported that the FG repeat nucleoporin Nup98 is enriched in P granules in embryos and is required for their integrity and perinuclear localization. *nos-2*, a translationally-repressed maternal mRNA enriched in P granules, dissociates from P granules and is prematurely translated in embryos lacking Nup98 (Voronina and Seydoux, 2010). Here we report that Nup98 is also required for P granule integrity and post-transcriptional silencing in adult germ cells.

We recently showed that mRNAs coding for meiotic structural proteins are expressed but silenced post-transcriptionally in germline stem cells and their progenitors (Merritt and Seydoux, 2010). Similar silencing has also been reported for the meiotic regulator GLD-1 (Jones et al., 1996; Crittenden et al., 2002). 3' UTR sequences are sufficient for silencing: transgenes containing a constitutive promoter driving GFP fused to the 3' UTRs of *gld-1*, *him-3*, *htp-2* or *syp-3* express GFP in germ cells that have initiated meiosis but not in the germline stem cells. We found that RNAi depletion of Nup98 activates GFP expression from these reporters in germline stem cells and their progenitors. Loss of P granule proteins has been shown to inhibit germline proliferation (Kawasaki et al., 1998; Kuznicki et al., 2000), but the molecular basis of this defect is not known. Our results suggest the intriguing hypothesis that disruption of P granules compromises germline stem cells by interfering with the silencing of mRNAs coding for meiotic proteins.

INVESTIGATING THE FUNCTION OF A NOVEL GERM-CELL SPECIFIC TARGET FOR CYCLIC NUCLEOTIDES

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Cyclic nucleotides are important second messengers that control a variety of physiological functions. In particular, cAMP and cGMP play major roles in sperm physiology. Whereas mainly cGMP controls sperm chemotaxis in marine invertebrates, cAMP regulates the swimming behaviour of mammalian sperm. However, the role of cyclic nucleotides during sperm-cell development is not well established. Only a few eukaryotic target proteins for cyclic nucleotides have been identified so far: the protein kinases A and G (PKA, PKG), the family of cyclic nucleotide-gated channels (HCN and CNG channels) and the exchange protein directly activated by cAMP (EPAC). Surprisingly, none of them seems to play a major role during spermatogenesis although the enzymes that synthesize cAMP and cGMP, the adenylate and guanylate cyclases, can be found in sperm precursor cells.

We performed a database survey searching for unknown proteins containing a cyclic nucleotide binding-domain (CNBD) and identified in a testis-specific mouse EST-database a new protein that does not belong to any of the known families of target proteins for cyclic nucleotides. We called this protein SCNBP (soluble cyclic nucleotide binding-protein). It can be found in up to 40 different species ranging from ciona to human. In mouse, there is only one isoform, which is exclusively expressed in male germ cells in late spermatocytes and spermatids. In *drosophila*, there are three isoforms and at least two of them seem to be exclusively expressed in male germ cells. We are now trying to elucidate the role of the SCNBP during germ-cell development by generating *Scnbp* knock-out mice and flies. *Scnbp*-deficient male mice are subfertile, whereas their female counterparts display normal fertility. At the moment, we are analysing whether testis morphology, spermatogenesis and/or sperm function is altered.

Binding of cyclic nucleotides to the mSCNBP has been demonstrated by using fluorescence techniques. Further studies will show what the binding affinities for either cAMP or cGMP are.

In summary, we have identified a novel target for cyclic nucleotides, which seems to play an important role for male fertility.

ASSESSING THE EFFECTS OF THE SYSTEMIC ENVIRONMENT ON AGING OF MALE DROSOPHILA GERMLINE STEM CELLS

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Drosophila male germline stem cells (GSCs) are a well-studied model for stem cell/niche function – individual stem cells are easily identifiable, and their dynamics can be readily quantified. Recent studies from a number of labs have described changes in these stem cells during aging. Stem cells are lost from the stem cell niche over time, but a replacement mechanism that may involve de-differentiation of gonial cells results in only a modest decrease in total stem cell number during aging. Additionally, GSC division rates slow during aging, which may be in part due to a misorientation of centrosomes prior to mitosis. The underlying factors that contribute to these changes, however, remain poorly understood. We have set out to develop a testis transplantation system to directly assess the effects of systemic factors, such as insulin-like peptides and steroid hormones, on these changes. We have found that, similar to what has previously been done in the ovary (Lin and Spradling, 1993), testes can be transplanted into a recipient abdomen, and we show that GSCs in transplanted testes live and continue to divide for at least 3 weeks. Using this system, we are conducting transplants of either young or old testes into either young or aged recipients, and assaying for stem cell number and division rate in the new systemic environment. Given that recent studies have implicated nutrient sensing and insulin signaling pathways as important in GSC function, we expect to find that the age of the systemic environment in which GSCs are placed will influence their function. We can then begin to use this system to begin to explore how either genetic factors (e.g. pro-aging genes) or environmental stresses (e.g. oxidative damage) might affect the systemic environment.

CYTOPLASMIC PARTITIONING OF P GRANULE COMPONENTS IS NOT REQUIRED TO SPECIFY THE GERMLINE IN *C. ELEGANS*

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Asymmetric segregation of P granules during the first four divisions of the C. elegans embryo is a classic example of cytoplasmic partitioning of germline determinants. To gain insights into this process, we performed an RNAi screen for regulators of P granule integrity and identified *pptr-1*. In a *pptr-1* mutant, P granules become unstable during mitosis, and P granule proteins and RNAs are distributed equally to germline and somatic daughter cells. However, despite equal partitioning of P granule components, *pptr-1* mutants retain the ability to specify the germline. *pptr-1* mutants are fertile, except at high temperatures. We conclude that asymmetric partitioning of P granule components during mitosis is not essential to specify germ cell fate, but may protect the nascent germline from stress.

DEFICIENCY IN MOUSE Y CHROMOSOME LONG ARM GENE COMPLEMENT IS ASSOCIATED WITH SPERM DNA DAMAGE.

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Mice with severe non-PAR Y chromosome long arm (NPYq) deficiencies are infertile in vivo and in vitro. We have previously shown that sperm from these males, although having grossly malformed heads, were able to fertilize oocytes via intracytoplasmic sperm injection (ICSI) and yield live offspring. However, in continuing ICSI trials we noted a reduced efficiency when cryopreserved sperm were used and with epididymal sperm as compared to testicular sperm. In the present study we tested if NPYq deficiency is associated with sperm DNA damage - a known cause of poor ICSI success.

We observed that epididymal sperm from mice with severe NPYq deficiency (i.e. deletion of nine-tenths or the entire NPYq gene complement) are impaired in oocyte activation ability following ICSI and there is an increased incidence of oocyte arrest and paternal chromosome breaks. Comet assays revealed increased DNA damage in both epididymal and testicular sperm from these mice relative to controls, with epididymal sperm the more severely affected. In all mice the level of DNA damage was increased by freezing. Epididymal sperm from mice with severe NPYq deficiencies also suffered from impaired membrane integrity, abnormal chromatin condensation and suboptimal chromatin protamination. It is therefore likely that the increased DNA damage associated with NPYq deficiency is a consequence of disturbed chromatin remodeling.

This study provides the first evidence of DNA damage in sperm from mice with NPYq deficiencies and indicates that NPYq-encoded gene/s may play a role in processes regulating chromatin remodeling during spermiogenesis and thus in maintaining DNA integrity in sperm. The study also provides important insights on the regulation of sperm genomic integrity after they are released from the testis, in the epididymis and in the oocyte after fertilization. In the context of other recently published work our study points to the possibility of an increased mutational load across generations as a consequence of assisted reproduction with sperm resulting from defective chromatin compaction during spermiogenesis or sperm subjected to cryopreservation.

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* The authors contributed to the work equally.

CYST STEM CELL DEVELOPMENT AND REGULATION OF GERMLINE STEM CELL MAINTENANCE DURING DROSOPHILA TESTIS FORMATION

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Establishment and maintenance of functional stem cells is critical for organ development and tissue homeostasis. Drosophila testis are among the best characterized systems for studying stem cell behavior, with GSCs and a second population of somatic stem cells, termed cyst stem cells (CySCs), localized to a discrete stem cell niche at the testes apex. Communication between the niche, GSCs, and CySCs regulates the balance between stem cell maintenance and differentiation. But how does such a complex system with multiple stem cell types form? Recent data show that functional, asymmetrically dividing GSCs are first established at ~22 hrs AEL during Drosophila testes morphogenesis (Sheng et al, 2009). This process correlates with coalescence of the testes stem cell niche, or hub, but timing and development of CySCs was not examined. Here we show that functional CySCs are present at the time of GSC establishment, and examine the process of CySC development with respect to hub formation. We also examine the role of stat gene function in regulation of both GSC and CySC maintenance and differentiation. Together, our observations indicate that a fully function GSC niche, with all the cell types present in adult Drosophila testes, has formed by the end embryogenesis. As this occurs at a rapid rate, just hours after onset of gonad formation, further analysis of testis morphogenesis will likely prove a useful model for the coordinated development of complex stem cell systems with multiple stem cell types, such as those found in human skin, hair and brain.

DYNAMICS OF OVULATION IN THE SOUTH AMERICAN PLAINS VIZCACHA, *LAGOSTOMUS MAXIMUS* (MAMMALIA, RODENTIA): MASSIVE OVULATION, OVULATION DURING GESTATION AND OOCYTE QUALITY.

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The South American plains vizcacha, *Lagostomus maximus* , is a hystricognathe rodent that displays the highest ovulation rate recorded for a mammal, reaching up to 800 oocytes per cycle. This massive polyovulation has been classically explained as the result of the highly convoluted ovary increasing the organ surface for ovulation. More recently, we have shown that apoptosis-dependent follicular atresia is suppressed due to the over expression of anti-apoptotic *BCL2* gene and little or no expression of pro-apoptotic *BAX*, leading to little restriction to germ cell proliferation and folliculogenesis. Despite massive ovulation, a few 8 to 12 embryos are implanted and only 2 of them are born after a 155-day long gestation. The remaining embryos are resorbed at mid gestation. Ovulation number has been examined in the early 70´s by Weir who also suggested the possibility of ovulation during gestation, an event recently reinforced by our fortuitous finding of an ovulating female at mid-gestation. We examined during the last two years, natural and induced ovulation rate, ovulation during gestation, and oocyte quality in females throughout the reproductive season from a natural population at Buenos Aires province (ECAS, Villa Elisa). 22 out of 53 females captured at the beginning of the reproductive season, displayed a natural ovulation mean of 154.5 ±18.67, a quite far number from the 800 value mentioned above. It is worth, however, to mention that when animals analyzed by Weir (N= 9) are re-analyzed not taking into account the only female showing 800 eggs, a mean value of 159.9 ±35.48 comparable to our result emerges. Induction of ovulation by the administration of eGC and hCG or PPLE yielded up to 284 eggs. A total of 13 females were trapped at mid-gestation; 10 of them showed ovaries plenty of ovulatory points, however a very low number of eggs were recovered from oviduct flushing. Histological inspection of these ovaries reflected a high proportion of oocyte-retained ovulatory follicles, indicating an active recruitment process rather than a true ovulation. A very poor oocyte quality was evident both in natural and induced ovulation regarding morphological integrity and chromosome segregation. Interestingly, up to 30% parthenogenetic spontaneous activation was found. Our results confirm massive ovulation and highlight the existence of follicle recruitment/ovulation at mid-gestation. Low quality of released eggs could arise from suppression of apoptosis-dependent atresia.

COORDINATION OF MEIOTIC PROGRESSION WITH HOMOLOGUE ALIGNMENT REQUIRES MOUSE HORMAD2.

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Mouse HORMAD2 is a meiosis-specific protein with significant sequence similarity to budding yeast Hop1 and to meiosis specific HORMA-domain proteins in other species. Yeast Hop1 is required for synaptonemal complex (SC) formation, efficient double strand break (DSB) formation and for the prophase checkpoint that monitors SC defects and DNA damage. In addition, Hop1 plays a crucial role in inhibition of DNA repair from sister chromatids/ inter-homologue bias.
Here, we present the analysis of meiosis in *Hormad2* knockout animals. Homozygous male mutants are sterile with arrest of gametogenesis in the first meiotic prophase. Female animals are fertile. Axial elements and full length SCs frequently form in the mutant. Nevertheless, spermatocytes fail to progress to diplotene stage. We examine DSB formation, DSB repair and meiotic silencing of unsynapsed chromosomes, to determine what defects lead to failure of meiosis in the absence of HORMAD2. Based on our observations we propose a role for HORMAD2 in meiotic prophase checkpoint mechanisms and discuss which aspects of the Hop1 paradigm can be extended to mammals.

STOCHASTIC SELF-RENEWAL AND DIFFERENTIATION OF MAMMALIAN SPERMATOGONIAL STEM CELLS

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Alternative cell fate specification controlled by differential extrinsic cues, as proposed by the niche model, has been the most influential paradigm guiding tissue stem cell studies. However, in mammalian seminiferous tubules, spermatogonial stem cells (SSCs) and their early-differentiated progeny are intermingled in an apparently uniform setting on the basement membrane. The apparent disengagement of alternative fate outcomes with the environment challenges a deterministic role of environmental cues in the fate specification of mammalian SSC progeny and raises the question whether mammalian SSCs possess an intrinsic capacity to specify both self-renewal and differentiation autonomously.

To address this question, we tested whether rat SSCs can give rise to both new SSC and differentiated progeny under a well defined homogeneous ex vivo culture system devoid of a niche. Our data showed that robust SSC self-renewal and differentiation occurs concurrently under the uniform ex vivo setting. Employing novel single cell assays, we observed that the fate bifurcation takes place side by side in twin daughter cells of single SSCs, even when they have been exposed to virtually identical microenvironments in culture. Moreover, a combination of quantitative experimental measurements and mathematical modeling indicates that the autonomous fate decision is stochastic, with constant probability. In contrast to the extrinsic cues driven paradigm, our data show that differential extrinsic cues are not mandatory for mammalian SSC self-renewal and differentiation, and thus point to a novel underlying mechanism that is cell-autonomous and stochastic. We believe our findings may have broad implications for stem cells research.

ROLE OF GERM CELL SPECIFIC GENE, *TDRD5*, IN SPERMATOGENESIS

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Many of mammalian tudor domain containing proteins including TDRD1, TDRKH, TDRD4/RNF17, TDRD6, TDRD7, TDRD8/STK31 and TDRD9 are involved in male germ cell development. Knockout analyses of these proteins were carried out in several groups and found that there are at least two targets of action during spermatogenesis. One is at embryonic gonad where TDRD1 and TDRD9 interact with MILI and MIWI2, respectively, and involved in silencing retrotransposable elements. Knockout mutants of these genes cause increased expressions of retrotransposons and double strand breaks on genomic DNA, resulting in the defect at spermatocyte development. The other is at spermiogenesis at spermatid where no direct mechanism is yet specified, but *Tdrd6* mutant testis showed no elongated spermatid and aberrant chromatoid body formations.

Here we show that TDRD5, another TDRD member that contains one tudor domain, specifically expressed at embryonic gonad and adult testis, and localizes very similar to TDRD1. Confocal microscopic observations of TDRD5-EGFP reporter mice revealed that TDRD5 started to form granular structures at the periphery of nucleus and co-localized with TDRD1 in the E15.5 male gonadal PGCs. In adult testis, TDRD5 expression was observed in the typeA spermatogonia, pachytene spermatocytes and early round spermatids at where TDRD5 co-localized with TDRD1 granules at all stages.

Tdrd5 knockout mice showed the male sterility and loss of elongated spermatids. Detailed analysis of *Tdrd5*$^{-/-}$ testis indicated that inter-mitochondrial cements at pachytene spermatocytes were recduced, chromatoid body at round spermatids was destructured, and acrosome formation was disrupted. Although *Tdrd5*$^{-/-}$ mice also showed increase of retrotransposable element expressions at embryonic gonad and spermatocytes, spermatocyte development was normal and no severe double-strand breaks were observed. Instead, *Tdrd5*$^{-/-}$ mice showed aberrant localizations of MIWI, TDRD6 and TDRD7 at late-pachytene stage spermatocytes and gene expressions for spermiogenesis were down-regulated as compared to wildtype.

These data demonstrate that TDRD5 is essential for spermiogenesis-related gene expressions and structural organizations in round spermatids.

THE DEAD-BOX RNA HELICASE VASA FUNCTIONS IN CELL CYCLE PROGRESSION

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Several genes expressed in germ cells are conserved throughout the animal kingdom and recently some of these genes were found to function more broadly than originally anticipated. Vasa is a widely conserved ATP-dependent RNA helicase that functions in germ cells and in many stem cells from cnidarian to mammals. Although implicated in translational regulation, the function of Vasa is poorly understood. Here we report a novel function of Vasa that may be a fundamental role of this protein. Vasa protein is present in all blastomeres of the early sea urchin embryo, which cleaves rapidly without G1 and G2 stages, and its abundance oscillates with the cell cycle. Vasa associates with the spindle and the separating sister chromatids at metaphase, and disappears after telophase. Inhibition of Vasa protein synthesis interferes with proper chromosome segregations, arrests cells at M-phase, and delays cell cycle progression. Cdk activity is necessary for proper localization of Vasa; Vasa and Cyclin B co-localize on the spindle during M-phase in vivo, and functionally interacts in vitro. In rapidly cleaving embryonic cells, Vasa is proposed to regulate the translation of mitotic cyclins on the spindle during M-phase in this developmental strategy of early, rapid cell cycling, and this role may reflect its ancient function.

FUNCTIONAL RECONSTRUCTION OF NANOS3 EXPRESSION IN THE GERM CELL LINEAGE BY A NOVEL TRANSGENIC REPORTER REVEALS DISTINCT SUBCELLULAR LOCALIZATIONS OF NANOS3

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Mutations of RNA-binding proteins such as Nanos3, Tial1, and Dnd1 in mice have been known to result in the failure of survival and/or proliferation of primordial germ cells (PGCs) soon after their fate is specified [around embryonic day (E) 8.0], leading to the infertility of these animals. However, the mechanisms of actions of these RNA-binding proteins remain largely unresolved. As a foundation to explore the role of these RNA-binding proteins in germ cells, we established a novel transgenic reporter strain that expresses Nanos3 fused with EGFP under the control of *Nanos3* regulatory elements. Nanos3-EGFP exhibited exclusive expression in PGCs as early as E7.25 and continued to be expressed in female germ cells until around E14.5 and in male germ cells throughout the fetal period with declining expression levels after E16.5. Nanos3-EGFP resumed strong expression in postnatal spermatogonia and continued to be expressed in undifferentiated spermatogonial cells in adults. Importantly, the *Nanos3-EGFP* transgene rescued the sterile phenotype of *Nanos3* homozygous mutants, demonstrating the functional equivalency of Nanos3-EGFP with endogenous Nanos3. We found that, throughout germ cell development, a predominant amount of Nanos3-EGFP co-localized with Tial1 (also known as Tiar) and phosphorylated eIF2α, markers for the stress granules, whereas a fraction of it showed co-localization with Dcp1a, a marker for the processing bodies. On the other hand, Nanos3-EGFP did not co-localize with Tdrd1, a marker for the intermitochondrial cements, in spermatogenic cells. These findings unveil the presence of distinct post-transcriptional regulations in PGCs soon after their specification, for which RNA-binding proteins such as Nanos3 and Tial1 would play critical functions.

A SEX-SPECIFIC DEVELOPMENTAL REGULATOR IN THE DROSOPHILA GERMLINE

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Success to sexual reproduction requires germ cells to distinguish and establish male and female sexual identities. Relatively little is known about how germ cells execute their sexual development programs, thus we conducted an in situ screen to identify genes with sex-specific expression patterns in the Drosophila embryonic gonad. A male-specific gene that emerged was Phf7, which contains a putative PHD motif known to recognize methylated histone H3K4 residues. Interestingly, its expression is restricted to gonads in adults, and it is enriched in undifferentiated germline in males compared to females. Phf7 exhibits several sex-specific characteristics. Females mutant for Phf7 show no loss in fecundity whereas mutant males produce 4-fold less progeny. This reduction in fecundity is due in part to increased germline apoptosis and degeneration of developing germline cysts. In addition, overexpression of Phf7 in germ cells leads to dramatic loss of germ cells in the females but not in males. This phenotype is also stage-specific: only undifferentiated female germ cells are susceptible. The severe germline loss is likely the result of germline masculinization, as supported by experiments testing the functions of Phf7 in gonads with germ cells and the surrounding soma of opposing sexes. XY germ cells in a feminized soma typically have poor viability due to germline-soma incompatibility, and this phenotype is partially alleviated upon loss of Phf7, likely due to germline feminization. Conversely, XX germ cells in a masculinized soma display strong germline loss, but ectopic expression of Phf7 can nurture germline development up to late stages of spermatogenesis. These results indicate that Phf7 is able to trigger a male germline program possibly through responding to epigenetic modifications in the genome.

REDISTRIBUTION OF USP26 ON MOUSE SPERM HEAD DURING EPIDIDYMAL MATURATION

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The spermatozoa emerging from the testicles are nonfunctional. They require a maturation process in the epididymis during which they undergo morphological, biochemical, and physiological changes to gain motility and the ability to fertilize an egg. Several sperm membrane proteins have been found to change conformation and/or localization during epididymal maturation. We now provide evidence for the redistribution of the germ cell -specific ubiquitin specific protease USP26 on the sperm head during its passage through the epididymis. In mouse testes, USP26 was detected by immunostaining at the blood-testis barrier, and the interface between Sertoli cells and post-meiotic germ cells. It exhibited deubiquitinating activities, implicating a role in the migration of germ cells along seminiferous epithelium. On fully developed spermatozoa, USP26 was found on the outside of the acrosome covering the frontal portion of the sperm head. However, in sperm isolated from the caudal epididymis, the acrosome no longer occupied the apical tip of the head where USP26 assumed a unique three arm structure. Staining of different epididymis sections as well as sperm isolated from various regions of the epididymis showed that the remodeling of acrosome and USP26 occurred in the front end (zones 1-2) of the caput region. Induction of acrosome reaction or vigorous sonication of the epididymal sperm had little effect on the USP26 structure, whereas brief trypsin treatment destroyed the USP26 signals without significantly damaging the shape of the apical tip. USP26 at the sperm head lacked detectable deubiquitinating activities. Its function remains to be elucidated.

THE ROLE OF POLYCOMB GROUP PROTEINS DURING PRIMORDIAL GERM CELL DEVELOPMENT

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Cellular identities are defined by distinct gene expression programs, which are based on the interplay between transcription factors and the epigenetic states of chromatin including post-translational histone modifications, DNA-methylation and chromatin associated proteins. Polycomb repressive complexes, PRC2 and PRC1, are epigenetic modifiers mediating histone modifications related to gene repression. PRC2 contains the histone methyltransferases, Ezh2 and Ezh1, that catalyse tri-methylation of histone H3 at lysine 27. PRC1 contains the RING proteins, Rnf2 and Ring1A, that mediate mono-ubiquitination of histone H2A at K119. Since PRC1 has the ability to bind H3K27me3, PRC1 is suggested to be targeted to sequences marked by PRC2. So far PRCs have been shown to play a key role for the regulation of many developmental regulators as well as the maintenance of multi-potency of stem cells.

Primordial germ cells (PGCs) are the first lineage of germ cells in mouse development. After being specified, PGCs proliferate and migrate into developing genital ridges. During this phase, PGCs are known to change their epigenetic states; DNA methylation decreases globally including at imprinting loci, while the level of PRC2-mediated H3K27me3 increases.

We study mice conditionally deficient for Rnf2 in PGCs to address the role of PRC1 during PGCs development. We observed that adult germ-line tissues in mutant mice were reduced in size. Especially in testis, we observed seminiferous tubules lacking germ cells. By analysing embryonic gonads, we found that the number of PGC-marker positive cells is reduced in *Rnf2* mutant embryos in the post-migratory phase of development. After migration is completed, PGCs eventually stop proliferating and start to differentiate in a sex-specific manner. Current transcriptional profiling experiments suggest that PRC1 might play an important role in maintaining cell identity of PGCs at the onset of sex differentiation. We are currently examining this possibility in more detail.

RETROTRANSPOSON LIFE CYCLE IN THE MALE GERMLINE

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Genome-wide remodeling of epigenetic profiles occur in primordial germ cells in mammals, including a loss of methylation at various transposable elements (TEs). This opportunistic window for TE activity is rapidly counteracted by a cooperative effort between the RNA interference pathway and the DNA methylation machinery to ensure the stable suppression of TEs in developing male germ cells. Dnmt3L, a DNA-methyltransferase co-factor, is required for TE methylation during spermatogenesis. A germline-specific class of small RNAs, the piRNAs (Piwi-interacting RNAs), promote the degradation of TE transcripts and also help establish TE methylation. Mutant male mice for *Dnmt3L* or *Miwi2* (a Piwi protein) show massive TE reactivation that leads to complete sterility. These models were instrumental in stressing the importance of epigenetic control of TEs.

Very little is currently known about the interactions that exist between the male germline and TEs, and in particular what cellular components facilitate/limit TE life cycle. To observe how TEs are regulated during male germ cell development, we have followed the expression pattern of various classes of TEs at different pre-natal (12.5, 14.5, 16.5, 18.5 dpc) and post-natal (1, 5, 9, 12, 15, 24 dpp) time points throughout spermatogenesis. We have studied the relative contribution of transcriptional mechanisms to TE silencing by studying wild-type and *Dnmt3L* mouse mutants. We now have a clear time line of TE activity throughout male germ cell development in both the wildtype and *Dnmt3L* mutant situation. Further studies will investigate the contribution of other restricting pathways to TE silencing by studying the *Miwi2* mutant line, and newly established mutant lines of germline TE reactivation.

IDENTIFICATION AND CHARACTERIZATION OF A FAMILY OF LONG NON-CODING RNAS PRESENT IN MALE GERM CELLS OF LIZARD

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A number of long non-coding RNAs (lncRNAs) have been identified, some of which have been shown to play important roles in genomic imprinting, transcriptional regulation, RNA processing, and protein translation. However, it is computationally challenging to identify homologs or relatives of lncRNAs as a consequence of weak sequence conservation. We are studying the evolution and function of *MALAT1*, an abundant lncRNA which is processed by the *tRNA* processing machinery into a 6.7 kb nuclear-retained lncRNA and a 61-nt cytoplasmic *tRNA*-like small RNA (mascRNA) (Wilusz et al. 2008 Cell 135, 919-932). Utilizing a covariance model, we built a consensus RNA secondary structure profile for the ~200 nt region around the junction of the 3' end of *MALAT1* and mascRNA (mascRNA module). We searched genomic databases using this profile and identified more than 150 genomic loci containing the mascRNA module in vertebrate genomes. *MALAT1* homologs were further defined using syntenic and EST information from lizard, frog, and fishes. Our preliminary data demonstrated that, despite its poor primary sequence conservation within different species, *MALAT1* has a conserved localization in nuclear speckles which suggests that *MALAT1* may have plastic structure-function constraints during evolution.

In addition, we identified 47 genomic loci, containing the mascRNA module, that are highly expressed in *Anolis Carolinensis* testis. These transcripts do not contain potential ORFs longer than 100 amino acids. We refer to these transcripts as testis- abundant non-coding RNAs (tancRNAs). In contrast to *MALAT1*, which is exclusively present in the nuclei of Sertoli cells, tancRNAs are specifically expressed during late stages of spermatogensis and localize to cytoplasmic chromatoid bodies. Studies are underway to functionally characterize tancRNAs in germ cell chromatoid bodies.

DROSOPHILA PIWI FUNCTIONS IN HSP90-MEDIATED SUPPRESSION OF PHENOTYPIC VARIATION

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Canalization describes an organism's ability to produce the same phenotype despite genotypic variations and environmental influences. In Drosophila, Hsp90, the Trithorax group proteins, and transposon silencing have been implicated in canalization. Despite this, molecular mechanism underlying canalization remains elusive. Here, we show that the piRNA pathway, but not siRNA or miRNA pathways, is involved in canalization. Furthermore, we isolated a protein complex composed of Hsp90, Piwi, and the Hsp70/Hsp90 Organizing Protein Homolog (Hop), and demonstrated the function of this complex in canalization. Our data indicate that Hsp90 and Hop regulate the piRNA pathway via Piwi to mediate canalization. Moreover, they point to epigenetic silencing of the expression of existing genetic variants and the suppression of transposon-induced new genetic variation as two likely mechanisms underlying piRNA pathway-mediated canalization.

ZIF-1 TRANSLATIONAL REPRESSION DEFINES A SECOND, MUTUALLY EXCLUSIVE OMA FUNCTION IN TRANSCRIPTIONAL REPRESSION IN *C. ELEGANS* GERMLINE BLASTOMERES

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In *C. elegans*, germ cell precursors are generated through four rounds of asymmetric divisions, beginning with the 1-cell zygote, P0, each giving rise to a transcriptionally repressed germline blastomere (P1-P4), and a somatic sister cell which soon becomes transcriptionally active. Transcriptional repression in P2-P4 has been shown to require PIE-1, a protein provided maternally in oocytes and asymmetrically segregated to all germline blastomeres.

We have shown previously that OMA proteins directly repress global transcription in P0 and P1 by sequestering from the nucleus TAF-4, a TATA-binding protein associated factor and a key component of TFIID. Soon after the first mitotic cycle, OMA-1 and -2 undergo developmentally-regulated degradation. Here we show that OMA proteins also repress transcription in P2-P4 indirectly, through a completely different mechanism that operates in oocytes. OMA proteins bind to both the 3' UTR of the *zif-1* transcript as well as the eIF4E-binding protein, SPN-2, repressing translation of *zif-1* mRNA in oocytes. *zif-1* encodes the substrate-binding subunit of the E3 ligase for PIE-1 degradation. Inhibition of *zif-1* translation in oocytes is critical to ensure maintenance of PIE-1 levels in oocytes and, as a consequence, PIE-1 levels in germline blastomeres. The OMA proteins, which are absent from P2-P4, indirectly promote transcriptional repression in these cells.

We also show that the two OMA protein functions are strictly regulated in both space and time by MBK-2 kinase-dependent phosphorylation, as the MBK-2 kinase itself is only activated following fertilization. Phosphorylation by MBK-2 facilitates OMA proteins binding to TAF-4 and simultaneously inactivates their function in repressing *zif-1* translation. Phosphorylation of OMA (and possibly other) proteins displaces SPN-2 from the *zif-1* 3' UTR, releasing translational repression. This ensures correct developmental timing for activation of the E3 ligase that will degrade the small amount of PIE-1 protein remaining in somatic blastomeres. We propose that MBK-2 phosphorylation serves as the developmental switch that converts OMA proteins from repressors of specific protein translation in the gonad to global transcriptional repressors in the earliest germline precursors. Both OMA protein functions, one acting directly in the earliest germline blastomeres and the other acting earlier, and indirectly, in the oocytes, together help guarantee transcriptional repression in all germline blastomeres.

SELECTIVE REGULATION OF PROTEIN TRANSLATION IN MOUSE SPERMATOCYTES

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In the mouse testis, endogenous signals promote germ cell exit from meiotic prophase and the meiotic divisions. Both MPF (metaphase promoting factor) and aurora kinases are implicated, but the proximal regulators have not been defined. Additionally, it is not known whether or how much of this critical step in mammalian meiosis is acutely regulated at the translational level. We have obtained genetic evidence that selective regulation of translation is required for exit from meiotic prophase. The ENU-induced *repro8* mutation was identified in a screen to uncover genes that control fertility in the mouse. It causes male-limited infertility, with failure of spermatocytes to promote both desynapsis of homologous chromosomes and exit from the pachytene stage of meiotic prophase. The *repro8* mutation is in the *Eif4g3* gene, which encodes multiple transcripts for the ubiquitously expressed eukaryotic translation initiation factor 4, gamma 3. Mutant germ cells exhibit normal prophase chromosome dynamics, and many proteins characteristic of the prophase to metaphase transition are not obviously depleted. Additionally, mutant spermatocytes respond in part to pharmacological induction of M-phase by phosphorylation of histone H3, under control of aurora kinases. However, although the protein is present, the kinase activity of the CDC2A catalytic subunit of MPF is dramatically reduced in mutant spermatocytes. Further, HSPA2, a chaperone protein for CDC2A kinase, is absent in mutant spermatocytes in spite of the presence of *Hspa2* transcript. This finding is consistent with the observation that the *repro8* phenotype is markedly similar to the *Hspa2* knockout phenotype. Thus, EIF4G3 is required for HSPA2 translation in spermatocytes, a finding that provides the first genetic evidence for selective translational control of meiotic exit in mammalian spermatocytes. Supported by the NIH, HD33816.

PROTEIN SYNTHESIS IN THE GERMLINE: TRANSLATIONAL FACTOR ISOFORMS SELECT MRNAS FOR MEIOSIS AND DIFFERENTIATION.

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Translational control of mRNAs is the predominant mode of gene regulation in animal germ cells. Only recently, however, has the role of translation initiation factors (eIFs) in such translational control been appreciated. Our labs are uncovering mRNAs specifically regulated by individual isoforms of eIF4E, the cap-binding protein of the initiation complex, in *C. elegans* germ cells. We have now shown that deficiency in single isoforms of eIF4E (e.g. IFE-1 or IFE-2) prevents efficient translation of small subsets of mRNAs unique to that isoform. These mRNAs encode proteins of critical function in oocyte and/or spermatocyte differentiation. As a consequence, loss of one eIF4E isoform causes a germ cell differentiation defect that is wholly different from the defect caused by another isoform deficiency. Loss of germline-specific IFE-1 led to inefficient translation of a few select mRNAs (e.g. *mex-1* and *oma-1*), a cytokinetic defect in spermatocytes, and limited production of mature oocytes. Loss of IFE-2, on the other hand, caused aberrant meiotic chromosome segregation and prevented meiotic crossover due to poor translation of a different set of mRNAs (*msh-4* and *-5*). Neither eIF4E deficiency resulted in loss of global protein synthetic activity or general growth capacity. Thus, eIF4Es appear to play function-specific roles in meiosis, late oogenesis and spermatogenesis. Each isoform selectively recruits a pool of germline-specific mRNAs to coordinate protein expression. The evidence points to a positive regulatory network of eIF4E-mediated translational control directing developmental competence during gametogenesis.

CYTOPLASMIC POLYADENYLATION COMPLEXES IN THE C. ELEGANS GERM LINE

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Posttranscriptional mRNA regulation plays a crucial role during germline and early embryonic development. Our previous work on the regulation of *C. elegans* germ cell fate decisions revealed a pivotal role for two non-canonical poly(A) polymerases (PAP), GLD-2 and GLD-4. Both enzymes are co-expressed in the cytoplasm and associate with P granules, implying a shared role between the proteins. Indeed, the accumulation of the oogenic tumour suppressor GLD-1 during early meiosis is dependent on both cytoPAPs; the activity of *gld-2* and *gld-4* together prevent *fbf/ccr-4*-mediated *gld-1* mRNA destabilisation. Both cytoPAPs require protein interaction partners for their enzymatic activity and to recognize mRNA targets. One shared cytoPAP component is GLD-3, a multi-KH domain-containing protein of the Bicaudal-C family. However, our genetic experiments suggest the existence of many more regulators that must influence the activity of each cytoPAP in time and space.

Our continuous efforts to unravel the composition and function of cytoPAP complexes lead us to the identification of CPB-3, as a new GLD-2/-3 cytoPAP component. CPB-3 is one of four worm orthologs of the cytoplasmic polyadenylation element-binding protein family from *Xenopus*. We will present genetic, biochemical and cell biological evidence that CPB-3 protein is part of the cytoPAP machinery. Furthermore, we will demonstrate that *cpb-3* mRNA itself is under the control of cytoPAP-mediated translational regulation, and we will discuss our current working model on a putative autoregulatory loop of *cpb-3* expression.

BICAUDAL-C AND CCR4 REPRESS NANOS EXPRESSION DURING *DROSOPHILA* OOGENESIS

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Bicaudal-C (Bic-C) functions to establish anterior-posterior polarity in the *Drosophila* oocyte via repression of mRNA translation. In wild-type oocytes, the Nanos (Nos) protein is restricted to the pole plasm through multiple levels of translational repression. We found that Bic-C binds to *nos* mRNA both *in vitro* and *in vivo* and participates in regulating *nos* translation during oogenesis. Unlike wild-type ovaries, where Nos is only detectable in the germarium and in late oogenesis, in *Bic-C* homozygous mutants Nos protein accumulates in the oocyte, starting as early as stage 4, and is also detectable in the nurse cells. In mid-oogenesis, the average polyA tail length of *nos* mRNA in *Bic-C* ovaries is greater than in wild-type controls. We also observe this in *twin* (*ccr4*) mutant ovaries, suggesting that Bic-C and CCR4 may regulate nos expression via deadenylation, as was previously established for *Bic-C* mRNA. Our results suggest that some *nos* mRNA may escape repression in the ovary. Intriguingly, preliminary results suggest that this defect may be distinct from a simple *nos* over-expression in the ovary.
Bic-C co-immunoprecipitates with Glorund (Glo), another *nos* repressor, and this interaction is almost completely dependent on the presence of RNA, suggesting that they may co-exist in the same *nos* ribonucleoprotein complexes. Embryos produced by *Bic-C/+* heterozygous mothers exhibit ectopic Nos, which may interfere with anterior morphogenesis. Therefore, Bic-C dependent Nos derepression may underlie the observed bicaudal phenotype.

DAZL REGULATION OF RNA PROCESSING IN MAMMALIAN GERM CELLS.

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Post-transcriptional RNA processing, including mRNA storage, editing, decay and translational control, is central to the precise timing and location of gene expression required for normal development. RNA processing plays a central role in the germline specification in many non-mammalian organisms including *Drosophila*, *C. elegans*, *Danio rerio* and *Xenopus laevis.* In these species, RNA/protein complexes termed "germ plasm" are localized to a particular cytoplasmic compartment of the oocyte. Upon the first cleavage divisions, the germ cell fate is bestowed onto the daughter cells carrying the germ plasm. In mammals, however, the germ cell fate is not specified by germ plasm but by extracellular signals in the embryonic micro-environment. Nevertheless, many of the germ plasm components in the non-mammalian species are not only conserved in mammals but are also essential for mammalian germline development, suggesting the importance of RNA processing in mammalian germ cells.
Deleted in Azoospermia-like (Dazl) is a germ cell specific RNA-binding protein conserved among vertebrates. Mice lacking Dazl are infertile in both sexes, and the loss of primordial germ cells begins in early embryonic stages. Several mRNA targets of Dazl have been identified, but the functions of these targets do not cover the full spectrum of phenotypes caused by Dazl deletion. To elucidate the molecular functions of Dazl, we have generated a Dazl-GFP fusion knock-in reporter mouse. Using both *in vitro* and *in vivo* approaches we have identified a new class of RNA-protein granules found uniquely in the pre-meiotic germline. We show here that the localization of Dazl to the processing body-like granules is important for mammalian germ cell specification and/or germline stem cell maintenance. With this novel germ cell reporter system, we were able to identify novel downstream effectors of Dazl, which include nuclear proteins involved in the regulation of chromatin states.

TDRD1 RECRUITS PIRNA TARGETS AND PIWI PROTEINS TO FACILITATE PIRNA AMPLIFICATION

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Piwi proteins function in a germcell-specific RNAi–like pathway in many species. Two *piwi* genes, *ziwi* and *zili*, are essential in zebrafish germline maintenance and involved in the biogenesis of piRNAs. In a Zili immunoprecipitation (IP) followed by Mass Spectrometric (MS) analysis, interestingly, many tudor domain containing proteins (Tdrd) were identified, including Tdrd1. We show that zebrafish Tdrd1 is germline-specific and localizes to nuage together with Zili. Tdrd1 is important for nuage formation, for recruitment of Zili into nuage structures and is required for germcell development. Nevertheless a homozygous mutant, *tdrd1*$^{fh244-/-}$, shows a weaker phenotype than either of the Piwi knock-outs. To understand the role of Tdrd1 in piRNA pathway, small RNAs from *tdrd1*$^{fh244-/-}$, wild-type siblings and Tdrd1 IP were deep-sequenced. We found that Tdrd1 is not essential for piRNA biogenesis but does facilitate the process. Tdrd1 not only associates with Piwi proteins that are loaded with mature piRNAs but also long RNA transcripts, which are largely mapped to known piRNA locations in the genome and have the ping-pong signatures, suggesting that these long RNA transcripts are piRNA targets. It has been reported that tudor domains form protein-protein interactions via binding to methylated arginine (R) or lysine (K) residues. Interestingly, MS analysis showed that Zili is rich in methylated R and K residues, and R68 in the Zili N-terminus is symmetrically dimethylated (sDM). Using synthetic Zili peptides, we specifically pull down Tdrd1 with peptide containing sDM R68 but not asymmetrically di- or un-methylated peptides. These peptide-bound Tdrd1 proteins bind to two populations of Piwi proteins: loaded and un-loaded with piRNAs, suggesting that Tdrd1 functions as a scaffold for the piRNA pathway by gathering piRNA-loaded Piwi proteins, piRNA targets and unloaded counterpart Piwi proteins. Therefore Tdrd1 is important for efficient interaction between different components in the piRNA pathway.

CHROMOSOME DYNAMICS DURING MEIOSIS IN *C. ELEGANS*

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The germline in adult *C. elegans* is a syncytial tissue containing proliferating nuclei, as well as nuclei at all stages of meiosis. Work from a number of labs has uncovered cell signaling, cell cycle regulation, and translational control mechanisms that contribute to the switch from mitotic proliferation to meiotic entry. My group has been investigating the changes in nuclear architecture and chromosome organization that enable the meiotic program of homolog pairing, synapsis, and segregation to occur.

In vivo imaging of meiotic chromosomes has revealed that their dynamic behavior changes abruptly upon meiotic entry. Special chromosome sites called Pairing Centers associate with the nuclear envelope. Following meiotic S phase, chromosomes initiate bursts of rapid, saltatory motion. These rapid motions require a link between Pairing Centers and the microtubule cytoskeleton, and the activity of cytoplasmic dynein. These motions are not directional, and are as likely to move homologous chromosomes apart as to bring them closer together. Motion along the nuclear envelope also becomes less constrained, resulting in each chromosome exploring a much larger area of the nuclear surface per unit time. Quantitative analysis and simulations indicate that the loss of constraint alone is sufficient to dramatically increase the rate of homolog pairing, consistent with our observations that rapid motions are dispensable for pairing.

We have found that the Polo-like kinase PLK-2 is dispensable for mitosis but is a key regulator of meiotic chromosome dynamics. Upon meiotic entry, PLK-2 associates with Pairing Centers at the nuclear envelope, but later in prophase it is targeted to the synaptonemal complex. Its activity promotes rapid chromosome motions, and is also required for proper assembly and disassembly of the synaptonemal complex. In addition, PLK-2 is essential for the function of a checkpoint that delays meiotic progression in response to unsynapsed chromosomes. Thus, PLK-2 coordinates several major steps in the meiotic program of chromosome interactions and homolog segregation.

ILLUMINATING FORMATION AND REGULATION OF CROSSOVERS DURING C. ELEGANS MEIOSIS

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Meiosis is the specialized cell division program by which diploid germ cells generate haploid gametes. Reduction in ploidy occurs at meiosis I, following an extended prophase in which chromosomes identify and align with their homologs, stabilize this arrangement through assembly of the synaptonemal complex (SC) and undergo crossover recombination between their DNA molecules. Crossovers at the DNA level collaborate with sister chromatid cohesion to form chiasmata, temporary links that hold homologs together after loss of side-by-side alignment and allow them to orient, and ultimately segregate, toward opposite poles of the meiosis I spindle. Thus, mechanisms that underlie the formation and regulation of meiotic crossovers are crucial to the successful formation of euploid gametes. Meiotic crossing over is accomplished by deliberate induction of double-strand DNA breaks (DSBs), followed by repair of these breaks using meiosis-specific modifications of DSBR pathways in the context of meiosis-specific chromosome architecture. This process is subject to multiple levels of regulation to ensure that DSBs are formed and repaired in an appropriate temporal and spatial context, both to avoid posing a threat to genome integrity and to guarantee that each chromosome pair will undergo the obligate crossover required to promote homolog segregation. We are investigating the mechanisms that regulate and execute meiotic crossing over and chiasma formation in the nematode *C. elegans*, a simple metazoan organism that is especially amenable to combining cytological and genetic approaches in a single experimental system, and in which robust crossover regulation mechanisms have been shown to operate. We have developed and implemented assays to monitor the outcome of double-strand break repair for DNA breaks induced in *C. elegans* germ cells at defined chromosomal sites and/or at distinct times during meiotic prophase progression. Further, we have identified COSA-1, a novel component of the crossover recombination machinery conserved in metazoans, and have shown that a functional GFP-tagged version of COSA-1 localizes to crossover sites. We will discuss insights into the mechanisms and regulation of meiotic crossing over revealed through the use of these experimental tools. We will also discuss the interrelationships between crossovers and the function, formation and remodeling of meiosis-specific chromosome structures.

CHROMATIN AND MEIOTIC CROSSOVER FORMATION

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Formation of meiotic crossovers is essential for proper chromosome segregation into developing gametes. Defects in crossover formation can lead to aneuploidy and developmental abnormalities. Meiotic crossovers are initiated by double strand breaks (DSBs) that occur preferentially in regions known as hotspots. The factors that contribute to hotspot formation and usage are poorly understand, although, recent advances have revealed a significant role for histone post-translational modifications in this process.

We have been analyzing two *C. elegans* genes, *xnd-1* and *him-5*, that are required for meiotic DSB formation on the X chromosome and for the global distribution of crossovers in the genome. These genes are of particular interest because they define the first mutations in any system that abrogate crossover formation on a single chromosome. Furthermore, because the sex chromosomes have unique developmental roles and expression patterns, understanding the functions of *xnd-1* and *him-5* may shed light on both crossover control and X chromosome biology.

Antibodies against Xnd-1 reveal that the protein is autosomally enriched, similar to Mes-4 and Mrg-1, two proteins required for X chromosome gene silencing. We have shown genetically that *xnd-1* is suppressed by *mes* mutants, suggesting that *xnd-1* is acting on germline chromatin. Furthermore, we have identified an increase in histone H2A lysine 5 acetylation (H2A K5Ac) in this mutant, which is suppressed by knocking down expression of the histone acetyltransferase *mys-1/Tip60*. In contrast to *xnd-1*, *him-5* mutants do not appear to have a significant affect of H2A K5Ac levels, suggesting that these genes are affecting crossover formation in fundamentally different ways.

THE STRUCTURAL NUCLEOPORIN SEH1 REGULATES THE MAINTENANCE OF OOCYTE IDENTITY AND THE CONSTRUCTION OF THE MEIOSIS I SPINDLE DURING *DROSOPHILA* OOGENESIS

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The nuclear pore complex (NPC) mediates transport of macromolecules between the nucleus and the cytoplasm. Recent evidence indicates that structural nucleoporins, the building blocks of the NPC, have a variety unanticipated cellular functions. We have defined a novel tissue specific requirement for the structural nucleoporin Seh1 during *Drosophila* oogenesis. Seh1 is a component of the Nup107-160 complex, the major structural subcomplex of the NPC. In vertebrates the Nup107-160 complex contributes to the construction of bipolar spindles. We determined that Seh1 physically interacts with the product of the *missing oocyte* (*mio*) gene. In *Drosophila*, *mio* regulates meiotic progression and the maintenance of oocyte identity. We find that like *mio*, the nucleoporin *seh1* has a critical germline function during oogenesis. In both *mio* and *seh1* mutants, a fraction of oocytes fail to maintain the meiotic cycle and develop as pseudo-nurse cells. In *seh1* ovarian cysts, the failure to maintain oocyte identity correlates with the aberrant organization of microtubules. Intriguingly, the fraction of *seh1* oocytes that remain in the meiotic cycle and continue oogenesis form highly aberrant meiosis I spindles. Surprisingly, our characterization of a *seh1* null allele indicates that while required in the female germline for proper execution of both the mitotic and meiotic divisions, *seh1* is dispensable for the development of somatic tissues. Our work represents the first examination of *seh1* function within the context of a multicellular organism.

REGULATION OF THE PROPHASE-TO-METAPHASE TRANSITION IN FOLLICLE-ENCLOSED MOUSE OOCYTES: TWO PATHS TO MEIOTIC RESUMPTION

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Mammalian oocytes are arrested in meiotic prophase by an inhibitory signal from the surrounding somatic cells in the ovarian follicle. In response to luteinizing hormone (LH), which binds to receptors on the somatic cells, the oocyte proceeds to second metaphase, where it can be fertilized. We investigated how the somatic cells regulate the prophase-to-metaphase transition in the oocyte, and showed that the inhibitory signal from the somatic cells is cGMP. Using FRET-based cyclic nucleotide sensors in follicle-enclosed mouse oocytes, we found that cGMP passes through gap junctions into the oocyte, where it inhibits the hydrolysis of cAMP by the phosphodiesterase PDE3A. This inhibition maintains a high concentration of cAMP, and thus blocks meiotic progression. LH reverses the inhibitory signal by lowering cGMP in the somatic cells (from ~2 μM to ~ 80 nM at 1 hour after LH) and by closing gap junctions between the somatic cells. The resulting decrease in oocyte cGMP (from ~1 μM to ~40 nM) relieves the inhibition of PDE3A, increasing its activity by ~5-fold. This causes a decrease in oocyte cAMP (from ~700 nM to ~140 nM), leading to the resumption of meiosis.

CDH1 CO-ORDINATES THE TIMING OF MEIOTIC RESUMPTION DURING PROPHASE I ARREST OF MAMMALIAN OOCYTES

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The Anaphase-Promoting Complex (APC) activator Cdh1 is recognized for its role in the mitotic cell cycle. In order to explore its function in female meiosis, we developed an oocyte-specific Cdh1 knockout mouse such that Cdh1 protein was absent in growing and mature oocytes. Loss of Cdh1 resulted in oocytes that had a propensity to undergo meiotic resumption out of prophase I arrest. This could be observed in: (1) growing oocytes, that normally have low competency to resume meiosis, (2) fully grown oocytes collected from antral follicles of hormonally primed animals, and (3) fully grown oocytes cultured in vitro. The maturation-inhibitory environment of the ovarian follicle acted to counterbalance the effects of Cdh1 loss, such that the greatest rates of meiotic resumption were observed in vitro on denuded oocytes. The inability of Cdh1 knockout oocytes to maintain a robust prophase I arrest was attributed to the 5-fold higher cyclin B1 levels, although we found that securin, another an APCCdh1 substrate, was completely unaffected in these oocytes. In summary, Cdh1 is required to keep cyclin B1 levels low in oocytes during the period of prophase I, and this activity is important in maintaining oocytes quiescent until a hormonal cue triggers meiotic resumption

AN ESSENTIAL ROLE FOR MOUSE MAELSTROM IN TRANSPOSON CONTROL IN THE FEMALE GERMLINE.

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Activation of transposable elements (TEs) is detrimental for genome integrity thus necessitating the existence of sophisticated defensive mechanisms regulating TE expression. In the mouse germline, TEs are repressed by a concerted action of DNA methylation and Piwi-interacting small RNAs (piRNAs). Genetic ablation of de novo DNA methylation or piRNA biogenesis precludes effiicient repression of TEs during epigenetic reprogramming in the embryonic germline leading to a failure of spermatogenesis. Paradoxically, the female germline appears to be refractory to the effects of TE upregulation since meiosis is initiated concurrently with TE derepression and no female germ cell phenotypes have been reported in mutants lacking piRNA components.

Here we present evidence that a null mutation in mouse Maelstrom (Mael), encoding an essentail factor of the piRNA pathway (Soper et al, Dev. Cell. 2008), perturbs normal progression of female meiosis and oogenesis due to transposon misregulation. Mael mRNA and protein are expressed in the female germline from primordial germ cells to the non-growing oocyte stage. In pure C57BL/6J mice, Mael mutation disrupts female meiosis leading to homologous chromosome asynapsis, lack of chiasma, germ cell loss, meiotic spindle defects and aneuploidy. Careful analysis of spatio-temporal expression of L1-encoded ORF1p protein revealed that the loss of Mael does not cause further derepression and upregulation of TEs at transcript or protein levels, but leads to misregulation of subcellular distribution of ORF1p. Specifically, in the Mael-mutant ovaries, we observe a ten-fold increase in the number of oocytes containing nuclear ORF1p compared to wild type. Our results suggest that, in the female germline, the genome's integrity and precision of meiosis are ensured not by means of transcriptional and translational control of TEs but by spatio-temporal regulation of TE subcellular localization. Taken together, our results reveal sexual dimorphism of transposon control and uncover an additional mechanism of regulation of transposon activity in the mouse germline.

AN 'INSIDE-OUT' KINETOCHORE-INDEPENDENT MECHANISM DRIVES ANAPHASE CHROMOSOME SEPARATION DURING ACENTROSOMAL MEIOSIS

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The self-organized assembly of acentrosomal meiotic spindles has been extensively studied but relatively little is known about the mechanisms directing segregation of meiotic chromosomes on these spindles. Kinetochores play an essential role in genome transmission by forming the interface between chromosomes and spindle microtubules. Two types of chromosome architectures are prevalent among eukaryotes: monocentric, in which localized centromeres restrict assembly of kinetochores to focused chromosomal sites, and holocentric, in which diffuse kinetochores assemble along the length of chromosomes. Holocentricity is widely prevalent in metazoans and is observed in distinct phyla such as lower plants, nematodes, and hemipteran insects. Here, we investigate the contribution of kinetochores during meiosis in the holocentric nematode *C. elegans*. We show that the conserved kinetochore protein KNL-1 directs assembly of meiotic kinetochores, whose activity orients chromosomes on the acentrosomal spindle. Surprisingly, in contrast to mitosis, chromosome separation during meiotic anaphase was kinetochore-independent. Instead, prior to anaphase separation, meiotic kinetochores and spindle poles disassembled along with microtubules on the poleward side of the chromosomes. During anaphase, microtubules were exclusively formed between the separating chromosomes. Functional analysis implicated a set of proteins that localize to a ring-shaped domain between the kinetochores in pre-anaphase spindle assembly and anaphase separation. Ring domain proteins are localized by the chromosomal passenger complex (CPC), whose local enrichment is patterned by recombination to control step-wise loss of meiotic cohesion. Thus, meiotic segregation in *C. elegans* is a two-stage process where kinetochores orient chromosomes but are dispensable for their separation. We suggest that separation is instead powered by an original 'inside-out' mechanism that is controlled by a meiosis-specific chromosomal domain to coordinate step-wise dissolution of cohesion with chromosome segregation.

LIVE IMAGING OF GERMLINE FOLLICLE FORMATION GIVES NEW INSIGHTS INTO GERMLINE-SOMA COORDINATION

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The Drosophila ovariole tip produces new ovarian follicles every 12 hours through the action of germline (GSC) and follicle (FSC) stem cells located within distinct niches. Static images provide a thumbnail view of three dimensional tissue structure and cellular gene expression, but capture dynamic cellular interactions to only a limited extent. We describe a live-imaging culture system that supports normal ovarian stem cell activity, cyst movement and follicle formation for at least 14 hours. Our studies revealed new insight into the role of the somatic escort cells, cells previously proposed to accompany differentiating germline cells and to be replenished by the division of distinct anterior stem cells (ESCs). Live imaging demonstrated that escort cells do not adhere to and migrate along with GSC daughters, but instead pass germ cell cysts from one escort cell to another in a complex process involving dynamic membrane activity. Nor do escort cells undergo regular mitosis; however, they divide readily in response to increases in the germ cell/escort cell ratio. Morphologically similar cells were visualized in the fetal mouse ovary. Our work identifies escort cells as a quiescent adult cell type that is capable of re-entering the cell cycle, identifies a pitfall of commonly used lineage marking systems, and provides new insight into soma-germline interactions that underlie early germ cell development in diverse organisms.

LIVE IMAGING OF THE *DROSOPHILA* TESTIS REVEALS DYNAMIC MECHANISMS OF STEM CELL LOSS AND REPLACEMENT

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Stem cells are thought to modulate their output by switching between symmetric and asymmetric divisions in response to cellular requirements during development or injury. Germline stem cells (GSCs) in the Drosophila testis adhere to a small cluster of somatic niche-generating cells called the hub, undergoing stereotypically oriented cell divisions which ensure an asymmetric outcome. Upon division, one GSC daughter that remains hub-associated and another called a gonialblast (GB) that is displaced away from the hub, and presumed to differentiate. However, stem cells undergoing asymmetric divisions alone cannot respond to perturbations causing stem cell loss, and mechanisms to replenish the stem cell pool exist to prevent eventual tissue failure. One such mechanism is the ability of differentiating stem cell daughters (spermatogonia) to revert into GSCs during aging and after genetically induced GSC loss. Here, live imaging of intact testes confirmed that the primary source of new GSCs following genetically-induced GSC loss was a select subset of four-cell spermatogonial cysts; these typically fragment into pairs while moving towards then establishing contact with the hub. Unexpectedly, although nearly all GSCs were constrained to divide perpendicularly to the hub, as in wild-type, GSCs also produced daughters that moved in to the niche and established contact with the hub. Interestingly, such GSC-GSC divisions were also captured during live imaging of young wild-type testes, and pulse-chase labeling and morphological analysis showed that that this event also occurred in vivo. FInally, a low level of GSC loss was also observed under all conditions. Thus, stem cell behavior in this niche is more dynamic than previously appreciated, and stereotypically oriented stem cell divisions are not strictly correlated with an asymmetric outcome in cell fate. Since both spermatogonial reversions and GSC-GSC divisions increased significantly in testes recovering from genetically induced GSC loss, the Drosophila testis will be useful for understanding how altered niche signals influence modes of stem cell renewal in vivo - an event hypothesized to occur but difficult to directly observe in most mammalian stem cell systems.

EVOLUTIONARY ANALYSIS OF THE *BAG OF MARBLES* GENE ELUCIDATES BOTH INTRASPECIFIC FUNCTION AND THE CONSEQUENCES OF INTERSPECIFIC DIVERGENCE

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Drosophila germline stem cells (GSCs) can both self-renew and differentiate to give rise to oocytes or sperm. The complex regulation underlying this process makes GSCs the evolutionary target of mutations and pathogens trying to ensure their transmission. We have shown that multiple GSC genes are experiencing rapid, adaptive protein evolution in *Drosophila melanogaster* and the closely related species, *D. simulans*, suggesting that it is beneficial for these proteins to accumulate amino acid changes. We have focused on one of these adaptively evolving genes, *bag of marbles* (*bam*), to understand the functional consequences of this adaptive evolution. The best characterized function of *bam* is initiating GSC differentiation in ovaries. *bam* also has additional roles in assembling the fusome, in regulating the number of cyst divisions, and in regulating e-cadherin expression. We are using interspecies complementation to test whether adaptive evolution of *bam* has caused detectable functional differences. Specifically, we have assayed the ability of a *bam* ortholog from *D. simulans* to complement the male and female sterility associated with a *bam* mutation in *D. melanogaster*. We have found that the *D. simulans bam* ortholog can complement male sterility but fails to fully complement the female sterility in *D. melanogaster*. The *D. simulans* ortholog can complement *bam*'s function in differentiation, but shows stem cell loss, improper number of cells/cyst, and mitotic synchrony. These data suggest that the evolutionary force driving the diversification of *bam* may be focused on the female germline, and we hypothesize this force may be conflict with bacterial endosymbionts due to their maternal inheritance and reproductive manipulation. The endosymbiont *Wolbachia pipientis* is an obligate, intracellular bacterium that has been shown to manipulate both the male and female germline in a variety of insects. To determine if any interaction existed between *bam* and *Wolbachia*, we tested the ability of *Wolbachia* to suppress *bam* hypomorphic mutants and found that the presence of *Wolbachia* can suppress *bam* female sterility. We also found suppression of female sterility in flies with *D. simulans* transgenic *bam* in our complementation assay. We are currently examining the nature of the interaction between *bam* and *Wolbachia* to try and identify the mechanism of suppression.

MITOCHONDRIAL QUALITY CONTROL IN THE DROSOPHILA OVARY

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Mitochondria are double-membrane organelles responsible for many biochemical functions in the cell, including making ATP. Mitochondria cannot be made de novo as they are complex and contain their own DNA, mtDNA. Therefore, mitochondria are maternally inherited, with all the embryo's mitochondria coming from the egg's cytoplasm. As mitochondria perform their normal cellular functions they can become damaged. Mitochondrial damage includes loss of the mitochondrial membrane potential, mutations to mtDNA, and increased production of reactive oxygen species. We are interested in how the female germline performs the important process of mitochondrial quality control, thus ensuring only highly functional mitochondria make it into the newly developing embryo. There are at least two basic mechanisms by which this can happen. One is to use mitochondrial transport and molecular motors in the germline cyst to sequester highly functional mitochondria into the oocyte and/or move damaged mitochondria out of the oocyte. The other is to target damaged mitochondria for destruction, termed mitophagy.

We have found the gene *clueless* is important for maintaining mitochondrial integrity in germ cells. In *clu* null mutant ovaries, mitochondria become mislocalized specifically to the plus-ends of microtubules and become swollen, indicative of damage. Clu protein is found throughout the cytoplasm, but is also in large particles surrounded by mitochondria. *clu* genetically interacts with the gene *parkin*, the fly homolog of PARK2, which in humans is mutated in half the cases of an inherited form of Parkinson's disease. Work pioneered by Narendra et al (JCB 183:795) found Parkin becomes specifically localized to damaged mitochondria, marking them for mitophagy. In *parkin* mutants, Clu protein is still present, but the particles are gone, and mitochondria become mislocalized, similar to *clu* mutants. In addition, we have found feeding flies paraquat to induce mitochondrial damage, or mutating mitochondrial superoxide dismutase, causes mitochondrial mislocalization. This evidence, taken together, suggests damaged mitochondria mislocalize in a specific way in female germ cells, and that Clu particles are biologically relevant for preventing mitochondrial damage, either by providing an important substrate to mitochondria, or by helping to target the normally low number of damaged mitochondria found in a healthy cell. *clu* has homologs in many species, including humans, and the fly mutant has a similar phenotype to *clu* mutant homologs found in Dictyostelium and Arabadopsis. Therefore, elucidating *clu* function during oogenesis will be important for understanding how germ cells control the quality of their mitochondria for the next generation.

ANALYZING P GRANULE DYNAMICS AND DETACHMENT IN THE *C. ELEGANS* GERMLINE.

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Germ cells in *C. elegans* contain unique organelles that are called P granules. Throughout most of the *C. elegans* life cycle, P granules are associated with clusters of nuclear pore complexes (NPCs) on germ cell nuclei, however, as germ cells differentiate into oocytes, P granules become cytoplasmic. Very little is known about how and why P granules transition between these two states (nuclear-associated versus cytoplasmic). To determine how P granules detach from the nucleus, we have performed live imaging of a P granule component (PGL-1) and a component of the cytoplasmic fibrils of NPCs (NPP-9) during the detachment process. Consistent with our previously reported immunofluorescence data, we see that detaching P granules retain NPP-9 association. The process of P granule detachment initiates with small spike-like projections of NPP-9 and leads to complete detachment within 8-10 minutes. The association of NPP-9 with detached P granules could suggest that either only outer components of the NPC detach with P granules or P granules pinch off with the entire nuclear envelope analogous to vesiculation of nuclear envelope during mitosis in Drosophila. To test that hypothesis, we localized the inner nuclear membrane protein SUN-1, and found that SUN-1 was not associated with detached P granules suggesting that P granules are not pinching off the nuclear membrane. Interestingly, our FRAP data suggest that NPP-9 dynamics correlate with the nuclear vs. cytoplasmic association of P granules. In the pachytene region where P granules are nuclear, NPP-9 is stable whereas in the oocytes, NPP-9 associated with cytoplasmic P granules is dynamic. These data show that after P granules sever from the NPC, new NPP-9 is recruited from the cytoplasm. Finally, to address the mechanism by which P granules detach, we are currently exploring the role of cytoskeletal interacting protein ZYG-12 in this process.

EXPLORING SPOROPTOSIS: A DEVELOPMENTALLY-PROGRAMMED NUCLEAR DESTRUCTION EVENT CONTRIBUTING TO YEAST GAMETOGENESIS

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The budding yeast Saccharomyces cerevisiae has long served as a favorite model organism for the study of fundamental aspects of eukaryotic cell biology. While yeast encode conserved proteins related to metazoan apoptosis, the existence of bona fide apoptotic biology in this organism has remained controversial. We have discovered that yeast execute a prominent nuclear destruction event during gametogenesis, effectively catabolizing immature gametes to aid in the development of their sister gametes. We have named this phenomenon “sporoptosis”, and as implied by its name, sporoptosis exhibits fascinating similarities with germline apoptosis occurring in animals.

Yeast gametogenesis, or sporulation, is triggered by nutritional stress, after which diploid cells exit the mitotic cycle and undergo meiosis to produce four gametes, or spores. Under conditions of severe carbon starvation, cells will produce four meiotic products but package only one or two of these into spores. This phenomenon is thought to be adaptive, balancing spore number with spore fitness. We have hypothesized that these unpackaged meiotic products are in fact actively catabolized.

To test this idea, we have performed live-cell fluorescence imaging experiments and documented a very striking and acute nuclear disassembly event where unpackaged nuclei appear to dissolve soon after meiosis. Complimentary biochemical approaches reveal that these disassembly events are accompanied by dramatic increases in the autophagic breakdown of nuclear protein. Intriguingly, cells undergoing nuclear destruction induce large-scale nuclear genome fragmentation in the form of nucleosomal ladders, a long-acknowledged hallmark of mammalian apoptosis. Moreover, we found the production of nucleosomal ladders to be completely dependent on the yeast homologue of endoG, a mammalian apoptotic nuclease which typically resides in mitochondria and translocates to the nucleus to fragment genomic DNA during cell death. Sporoptosis may represent a primordial function for cell death pathways in gamete development, being an example of a unicellular organism sacrificing proto-gametes to ensure the survival of its "germline". This resonates with the role of programmed cell death in eliminating unneeded cells during germline development in metazoans. Our ongoing work is focused on further genetic, molecular, and cell biological characterization of sporoptosis, with an emphasis on autophagic pathways.

MATERNAL TO EMBRYONIC TRANSTION IN MICE: A TALE OF TWO MATRICES

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The interregnum between transcription in fully-grown oocytes and activation of the embryonic genome dictates a role for maternal genes in early mouse embryogenesis. For successful development, eggs must be fertilized by a single sperm and cleavage-stage embryos must survive oviductal passage to implant on the uterine wall. Failure to prevent polyspermy or protect the dividing embryo results in embryonic death. The maternally encoded zona pellucida (ZP1, ZP2, ZP3) is an extracellular matrix that surrounds pre-implantation embryos and plays a major role in these two biological imperatives. Following fertilization, cortical granules fuse with the plasma membrane and the subsequent cleavage of ZP2 suggests release of a protease. We have biochemically identified the cleavage site on ZP2 as well as a candidate cortical granule protease (*Cgp*). The absence of the protease (Cgp^{Null}) or the presence of mutant ZP2 that cannot be cleaved ($Zp2^{Mut}$) result in de novo sperm binding to two-cell embryos with intact ZP2. Although fertile, Cgp^{Null} and $Zp2^{Mut}$ females have decreased fecundity with early embryonic loss. Taken together, these data suggest that post-fertilization ZP2 cleavage blocks polyspermy and provides increased structural integrity to the zona pellucida for protection of the pre-implantation mouse embryo.

While screening for oocyte-specific maternal effect genes, we identified a second matrix, the **s**ub**c**ortical **m**aternal **c**omplex (SCMC), that assembles during oocyte growth and is essential for progression beyond the first embryonic cell division. Using a combination of co-immunoprecipitation and mass spectrometry, four maternally encoded proteins that participate in the ~1 MDa complex have been detected. FLOPED, MATER and TLE6 interact with each other while Filia binds independently to MATER. First formed in the subcortex of growing oocytes, the SCMC is reversibly excluded from cell-cell contacts in the early embryo. This results in localization of the SCMC to the outer cells of the morula and blastocyst which suggests a role in establishing the trophectoderm lineage in early development. Targeted mutations of the single copy genes encoding individual components result either in arrest at the two-cell stage (*Floped*, *Mater*) or poor cell-cycle progression with chromosome aneuploidy and decreased fecundity (*Filia*). Thus, the SCMC ensures high quality cell cycle progression in preimplantation mouse development and may play a role in the initial establishment of embryonic cell lineages. Additional maternal effect genes are likely to further enable egg to embryo transitions by removing maternal detritus (RNA, protein), activating the embryonic genome and initiating cell division.

IN VITRO PRODUCTION OF FUNCTIONAL SPERM IN CULTURED NEONATAL MOUSE TESTES

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Spermatogenesis, from spermatogonial stem cells, through meiosis, to sperm formation, has never been reproduced in vitro in mammals, nor in any other species with a very few exceptions in some particular types of fish. Using organ culture method with serum free media, we show that neonatal mouse testes which contain only gonocytes or primitive spermatogonia as germ cells can produce sperm in vitro. The obtained sperm resulted in healthy offspring through microinsemination. In addition, neonatal testis tissues were cryopreserved and, after thawing, showed complete spermatogenesis in vitro. Our organ culture method could be applicable through further refinements to a variety of mammalian species. In particular, cryopreservation of the testis tissue of pediatric cancer patients could become a practical modality to ensure future fertility.

SEXUAL DEVELOPMENT OF THE GONAD AND GERM CELLS

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Sex determination is a critical decision during development that has profound consequences on animal morphology and behavior. A great deal of emphasis has been placed on how this decision is made in the soma, however, sex determination in the germline, allowing the production of sperm or egg, is perhaps the key sexual decision made in any species. Sex determination in the germline is usually regulated differently than in the soma, but less is known about how this decision is controlled. The soma plays a key role in influencing sex determination in the germline, but in many animals, such as mammals and Drosophila, the sex chromosome constitution of the germline is also important.

In Drosophila, sexual dimorphism in the germline is apparent as soon as the gonad forms during mid-embryogenesis when germ cells associate closely with the somatic gonad. Interactions between the somatic gonad and germ cells are critical for determining germline sex; sex-specific signals from both the male soma (through the Jak/Stat pathway) and the female soma (through an unknown pathway) control sex-specific gene expression in the germ cells. Later, interactions with the soma remain critical as germ cells undergo further sex-specific development into male or female germline stem cells and gametes. However, interactions with the soma are not sufficient to allow proper germ cell development; neither XY germ cells in a female soma nor XX germ cells in a male soma can develop properly as gametes.

Our lab is taking a number of approaches to understand how somatic influence and germ cell autonomous cues combine to control germline sex determination and sex-specific development. We are studying the role that sex determination in the soma plays in controlling sex-specific germ cell development. We are studying the role of genes that are sex-specifically expressed in the germ cells as well as the role of sex-specific alternative RNA splicing in the germline. We are also studying how the germ cell's own sex chromosome constitution contributes to germline sex determination. Our current work in some of these areas will be presented.

FGF9 SUPPRESSES FEMALE AND PROMOTES MALE GERM CELL FATE DURING GONADAL DEVELOPMENT IN MICE

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In the mouse fetal gonad, germ cells commit to a female or male sexual fate on the basis of environmental cues, rather than XX or XY chromosome constitution. Germ cells in a developing ovary enter into meiosis, hence committing to the female fate or oogenesis. In a developing testis, germ cells do not enter meiosis during fetal life but they do stop proliferating and arrest in G0/G1 (mitotic quiescence), hence committing to the male fate or spermatogenesis. In recent years we and others showed germ cells in a female mouse embryonic gonad are triggered to enter meiosis by the potent signaling molecule retinoic acid (RA). RA induces germ cells to express a key gene, *Stra8*, which encodes a protein essential for initiation of meiosis. In the developing testis, germ cells avoid entering meiosis because RA is actively degraded by a cytochrome P450 enzyme, CYP26B1. Hence, CYP26B1 is a 'meiosis-inhibiting' substance by virtue of its ability to degrade the meiosis inducer, RA. There is evidence, however, for an additional secreted testis-specific meiosis-inhibiting factor.

Fibroblast growth factor 9 (FGF9) is produced by pre-Sertoli cells and has a known and important role in somatic sex determination: deletion of *Fgf9* leads to male-to-female sex reversal. In this study, we find that FGF9 also plays a critical and direct role in germ cell sex determination. We show, using *ex vivo* gain- and loss-of-function studies in gonads and isolated germ cells, and *in vivo* analysis of *Cyp26b1*-, *Fgf9*- and double-knockout embryos, that FGF9 acts directly on germ cells to antagonise their entry into meiosis, making them less responsive to RA. We show that FGF9 signalling plays a further role, maintaining pluripotency of germ cells and actively promoting a male fate. Our data suggest a model whereby germ cell sexual fate is determined by the relative abundance of FGF9 and RA. Such a system, incorporating both positive and negative regulatory cues, imparts stability at the crucial stages of germ cell sexual determination.

GENETIC ANALYSIS OF THE *DMRT1* GENE IN ZEBRAFISH SEX DETERMINATION AND GONAD DEVELOPMENT

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The *dmrt1* (*doublesex and mab-3 related transcription factor 1*) gene is important for sex determination and/or gonadal sex differentiation in many animals from invertebrates to vertebrates. This is unusual given that sex determination genes are typically not well conserved. In medaka, a recently duplicated copy of *dmrt1*, called *DMY* or *dmrt2by*, is the master male determining gene on the Y chromosome. The mechanisms by which zebrafish sex is determined has remained elusive due to the lack of sex chromosomes and the complex nature of sex determination in this organism. We are studying the role of *dmrt1* in sex determination and gonad sex differentiation in zebrafish. Expression of *dmrt1* was observed in adult ovaries, primarily in previtellogenic oocytes, and in the testicular Sertoli cells, spermatogonia and spermatocytes. In juvenile fish at stages when the gonad was differentiating into ovary or testis, *dmrt1* RNA was detected only in developing testes. To investigate the role of *dmrt1* in sex determination and gonad development, we isolated mutations disrupting this gene. The first, $dmrt1^{S10X}$, results in a stop codon after the 9th amino acid. The second, $dmrt1^{ins}$, is a retroviral insertion that disrupts the locus at position +7. In $dmrt1^{S10X}$ mutants, mutant RNA is made and likely a protein product, possibly initiated from an in-frame downstream ATG. In $dmrt1^{ins}$ mutants, no RNA was detected therefore this is a putative null allele. Fish homozygous for the null allele showed no alteration of sex-ratios when compared to wild-type siblings, indicating that *dmrt1* is not necessary for sex determination. However, $dmrt1^{S10X}$ lead to female biased sex ratios in fish that were either heterozygous or homozygous, with homozygotes having a stronger bias. Thus, the mutant protein is either interfering with male sex determination or promoting female sex determination. We found that Sertoli cells and germ cells were absent in testes of adult $dmrt1^{ins}$ whereas females were unaffected. In summary, we found that *dmrt1* is essential for testis development and male fertility while dispensable for ovary development. Furthermore, *dmrt1* is not required for sex determination, however the mutant protein produced by the $dmrt1^{S10X}$ allele perturbs the sex determination system suggesting that *dmrt1* may play a role in this process. Effects on sex determination by the null mutation may be masked by redundancy, possibly with other *dmrt* genes expressed in the gonads.

MICROENVIRONMENTAL CONTROL OF STEM CELL POPULATION IN MOUSE SPERMATOGENESIS

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In the steady-stage mouse spermatogenesis, GFRα1+ (also Nanos2+) spermatogonia convey the highest probability of self-renewal and mostly represent the actual stem cell compartment. While maintaining themselves, GFRα1+ cells give rise to Ngn3+ population, which is primed for differentiation but capable of reverting into being GFRα1+ and actively contributing to the stem cell pool maintenance.
Preceding live-imaging analyses revealed that Ngn3+ spermatogonia are biased to area adjacent to the blood vessels, before spreading over the tubules upon differentiation into Kit+ A1 spermatogonia. Recently, our success in live-imaging the GFRα1+ spermatogonia has revealed that some of them seem to prefer the vascular region, while others are moving around the tubules. The prominent spermatogonial migration looks compatible to the rapid replacement of neighboring stem cells in the tubules, drawn statistically from the fates of pulse-labeled stem cells.
In addition to the yet-to-investigate effect to the GFRα1+ population, the vasculature-associated region provides the site of GFRα1+ to Ngn3+ transition and residence of Ngn3+ cells before becoming Kit+ A1 spermatogonia, which are no more attracted by this region. This has motivated us to investigate the molecular and cellular functionality of this unique ‘stem cell niche’. Microdissection followed by microarray analyses have suggested a specialization of somatic support cells in this vasculature-associated region.

TGFβ SIGNALING IN THE *C. ELEGANS* NICHE REGULATES GERM CELL FATE

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Germline development requires precise spatial and temporal regulation of cell proliferation and cell fate determination to assure a reproductively competent individual. In the *C. elegans* germ line, GLP-1/Notch signaling is important for maintaining a proliferating population of germ cells: loss of *glp-1* activity causes all germ cells to differentiate. We have shown that Notch-independent signaling pathways such as insulin/IGF contribute to the robust proliferation of the larval germ line that is required for optimal fecundity.

To identify additional genes and pathways required for robust larval germline proliferation, we performed a genome-scale post-embryonic RNAi modifier screen. We developed a genetic strategy to identify genes required for the developmental control of germline proliferation/differentiation, rather than the many genes required for cell proliferation *per se*. We identified genes that cover a broad spectrum of families and functions that give us a more extensive knowledge of the control of germline proliferation.

Of particular interest, we found a novel role for the TGFβ signaling pathway in the niche to promote the undifferentiated fate in conjunction with the Notch pathway.

THE STEROID HORMONE ECDYSONE FUNCTIONS WITH INTRINSIC CHROMATIN REMODELING FACTORS TO CONTROL FEMALE GERMLINE STEM CELLS IN DROSOPHILA

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Steroid hormones are known systemic regulators of multiple tissues; however, whether or how they impact the fate and function of adult stem cells is unclear. In the Drosophila ovary, insulin signals modulate the proliferation and self-renewal of germline stem cells (GSCs), yet despite evidence that additional systemic factors control GSC activity, these have remained largely unknown. Our results show that ecdysone, a steroid hormone produced in later developing follicles, directly regulates adult GSC proliferation and self-renewal independently of insulin signaling. Ecdysone controls GSCs through a functional interaction with the chromatin remodeling factors ISWI, an intrinsic epigenetic factor required for GSC fate and activity, and Nurf301, the largest subunit of the ISWI-containing NURF chromatin remodeling complex. We propose the model that ecdysone produced by more differentiated ovarian cells under favorable conditions acts on GSCs as part of a positive feedback mechanism. Our findings support a link between systemic steroid hormones and the intrinsic chromatin remodeling machinery as a potential mechanism to promote broad transcriptional programs required for adult stem cell self-renewal.

HORMONAL COORDINATION OF NICHE FORMATION WITH GERM LINE STEM CELL ESTABLISHMENT.

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In a Drosophila ovary, somatic niches and germ line stem cells (GSCs) form functional units that maintain oogenesis throughout the lifetime of the organism. We have found that Ecdysone signaling during larval development is required for the correct formation of these stem cell units and for the initiation of oogenesis.
The Ecdysone receptor (EcR) and its co-receptor Ultraspiracle (Usp) are expressed in the somatic cells of larval ovaries. At early larval stages, EcR and Usp act as repressors of somatic niche formation. Indirectly, EcR and Usp also repress precocious differentiation of primordial germ cells (PGCs), the precursor cells for GSCs. This early repression of differentiation allows both niche precursor cells and GSC precursors time to proliferate.
Similar to imaginal discs, EcR and Usp act at early larval stages as repressors of at least one Broad isoform: Broad-Z1. Reduction of either EcR or Usp expression by RNAi leads to precocious expression of Broad-Z1, precocious niche formation, and precocious PGC differentiation.
At late larval stages, with rising levels of Ecdysone, EcR and Usp are required for Broad-Z1 expression, for niche formation, and for PGC differentiation. Expression of a dominant negative form of EcR (EcRA.W650A) leads to ovaries containing no niches. PGCs fail to differentiate in these ovaries. Significantly, while PGCs in EcRA.W650A do not differentiate, and do not express the major differentiation gene *bag of marbles* (*bam*), they also do not localize phosphorylated Mad in their nuclei. Thus, the major pathway that controls the maintenance and differentiation of GSCs/PGCs requires input that depends on somatic Ecdysone signaling.
We propose that Ecdysone receptors are major coordinators of ovary formation by their dual role as repressors, and later as activators of *broad* expression. Ecdysone signaling in the ovary is one example of a single signaling pathway controlling niche formation, establishment of its resident stem cells and the initiation of their differentiation.

DNA-SEQUENCE-BASED ANALYSES OF MAMMALIAN X AND Y, AVIAN Z AND W: CHROMOSOMES WITH PROMINENT ROLES IN GERM CELL DEVELOPMENT

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The mammalian X and Y chromosomes evolved from an ordinary pair of autosomes that existed in a reptilian ancestor that likely relied upon temperature-dependent sex determination, as in crocodiles today. Independently and concurrently, the avian Z and W chromosomes (ZZ males, ZW females) evolved from a different pair of autosomes that was present in the same ancestor. Both the mammalian XY pair and the avian ZW pair have emerged with specialized and disproportionate roles in germ cell development. These germ cell specializations are best understood in the case of mammalian Y chromosomes and are only now being appreciated in the case of mammalian X chromosomes and the avian ZW pair.

To reconstruct and better understand nature's sex chromosome experiment, we have set out to comprehensively sequence and compare the sex chromosomes of four primates, two rodents, an ungulate, a marsupial, and a bird. I will describe insights that are now emerging from this ongoing effort.

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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 64515 (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
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