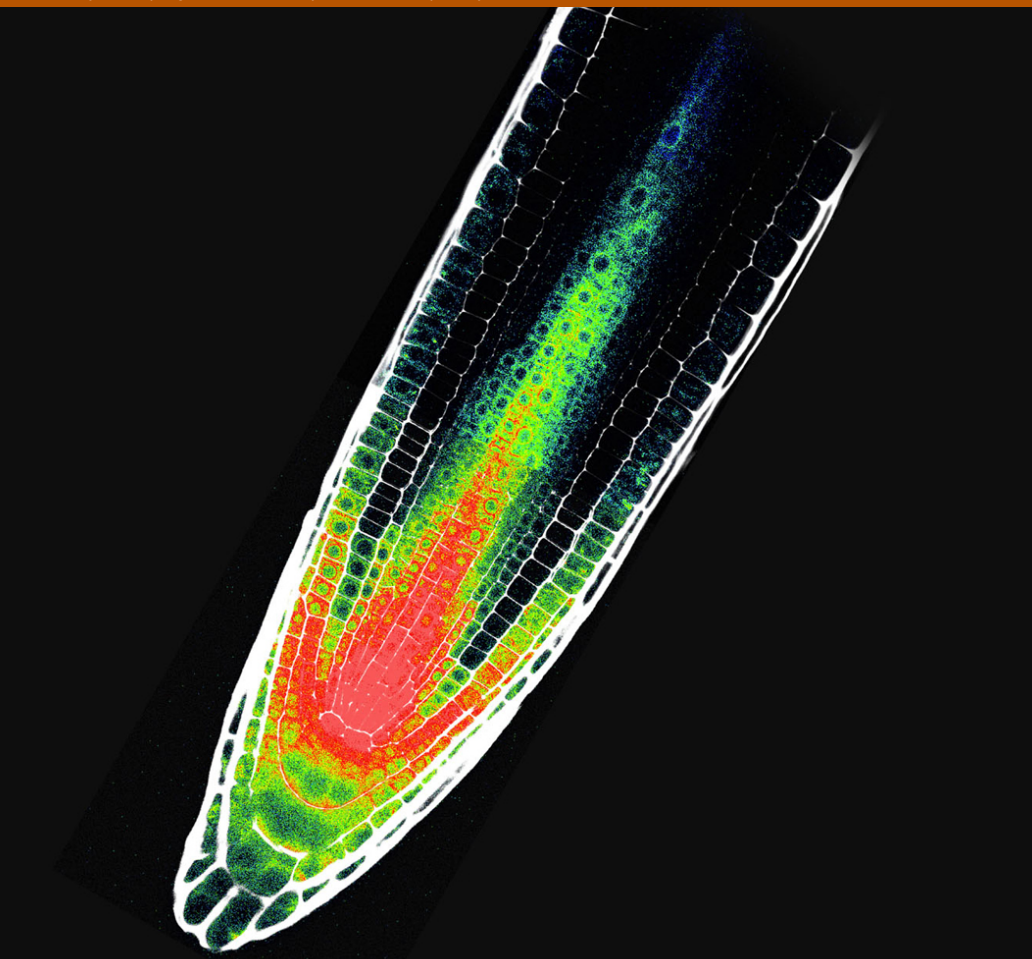
Joint Cold Spring Harbor Laboratory/Wellcome Trust Conference

COMPUTATIONAL CELL BIOLOGY

February 10–February 13, 2010



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COMPUTATIONAL CELL BIOLOGY

February 10–February 13, 2010

Arranged by

Dennis Bray, *University of Cambridge, UK*
Nicolas Le Novere, *EMBL-European Bioinformatics Institute, UK*
Leslie Loew*, University of Connecticut Health Center, USA*

CONFERENCE CENTRE
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SCHEDULE AT A GLANCE

Wednesday 10th February 2010

15.00-18.00	Registration
18.00-19.00	Keynote Speaker: Jim Ferrell
19.00-19.30	Informal Gathering and Cash Bar
19.30-21.00	Dinner

Thursday 11th February 2010

07.30-09.00	Breakfast
09.00-11.00	Session 1: Intracellular Signalling
11.00-11:30	Coffee Break
11:30-12:30	Session 1, continued
12.30-14.00	Lunch
14.00-15.30	Session 2: Tools and Software
15.30-16.00	Coffee Break
16.00-17.30	Session 2, continued
17.30-19.30	Software Demo / Poster Session I and Wine & Cheese Party
19.30-21.00	Dinner

Friday 12th February 2010

07.30-09.00	Breakfast
09.00-11.00	Session 3: Self-organization in Cells
11.00-11.30	Coffee Break
11.30-12.30	Session 3, continued
12.30-14.00	Lunch
14.00-15.30	Session 4: Cell Motility
15.30-16.00	Coffee Break
16.00-17.30	Session 4, continued
17.30-19.30	Software Demo / Poster Session II
19.30-21.00	Dinner

Saturday 13th February 2010

07.30-09.00	Breakfast
09.00-10.00	Keynote Speaker: Ben Scheres
10.00-11.00	Session 5: Patterns in Development
11.00-11.30	Coffee Break
11.30-13.30	Session 5, continued
13.30-14.30	Lunch
14.30-16.00	Session 6: Cell Biophysics
16.00-16.30	Coffee Break
16.30-18.00	Session 6, continued
18.00-19.30	Pre-dinner Drinks
19.30-21.00	Conference Dinner

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Cover: Computer model of a root tip. Ben Scheres, Utrecht University.

PROGRAM

WEDNESDAY, February 10—6:00 PM

KEYNOTE SPEAKER

James Ferrell
Stanford University

"Cooperative phosphorylation in the regulation of Wee1A" 1

THURSDAY, February 11—9:00 AM

SESSION 1 INT RACELLULAR SIGNALLING

Chairperson: **M. White,** University of Liverpool, UK

White, M., School of Biological Science, University of Liverpool, United Kingdom: Spatial and temporal information coding by the NF-κB system. 2

Cohen-Saidon, C., Cohen, A.A., Sigal, A., Liron, Y., Alon, U., Dept. of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel: Dynamics and variability in ERK2 response in individual living human cells. 3

Cuninkova, L., Moriggi, E., Brown, S.A., Institute of Pharmacology and Toxicology, University of Zurich, Switzerland: Molecular biomarkers for individual differences in human signaling cascades. 4

Plant, N.,[1] Kolodkin, A.,[2] Bruggeman, F.,[2] [1]Centre for Toxicology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom; [2]Regulatory Networks Group, Netherlands Institute for Systems Biology, Life Sciences, CWI, Amsterdam, The Netherlands: Nuclear receptors as controlling factors in chemical metabolism—Determination of interaction networks crucial for coordinating cellular response to chemicals. 5

Csikász-Nagy, A., Mura, I., Centre for Computational and Systems Biology, Microsoft Research, University of Trento, Italy: Role of mRNA gestation and senescence in noise reduction during the cell cycle. 6

SESSION 2 TOOLS AND SOFTWARE

Chairperson: **N. Le Novère,** EMBL-European Bioinformatics Institute, Hinxton, UK

THURSDAY, February 11—5:30 PM

SOFTWARE DEMO AND POSTER SESSION I
and WINE & CHEESE PARTY

Software Demos:

Posters:

FRIDAY, February 12—9:00 AM

SESSION 3 SELF-ORGANIZATION IN CELLS

Chairperson: **B. Mulder,** FOM Institute AMOLF, Amsterdam, The Netherlands

FRIDAY, February 12—2:00 PM

SESSION 4 CELL MOTILITY

Chairperson: **F. Nédélec,** European Molecular Biology Laboratory, Heidelberg, Germany

FRIDAY, February 12—5:30 PM

SOFTWARE DEMO AND POSTER SESSION II

Software demos:

Posters: *See Poster Session I for list of posters*

SATURDAY, February 13—9:00 AM

KEYNOTE SPEAKER

Ben Scheres
Utrecht University

SATURDAY, February 13—10:00 AM

SESSION 5 PATTERNS IN DEVELOPMENT

Chairperson: **J. Traas,** École Normale Supérieure de Lyon, France

SATURDAY, February 13—2:30 PM

SESSION 6 CELL BIOPHYSICS

Chairperson: **K. Kruse,** Universität des Saarlandes, Germany

SATURDAY, February 13—7:30 PM

CONFERENCE DINNER

COOPERATIVE PHOSPHORYLATION IN THE REGULATION OF WEE1A

Sun Young Kim and James E. Ferrell, Jr.
Dept. of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA

The proteins CDK1, Wee1, and Cdc25 collectively form a bistable switch that acts as a trigger for mitosis. One subcircuit of the switch is the double-negative feedback loop between CDK1 and Wee1; active Wee1 phosphorylates Y15 in CDK1 and thereby inactivates it, and active CDK1 phosphorylates many sites in Wee1 and eventually inactivates it. Here we investigated the mechanism through which the interaction of Wee1 and CDK1 flips from one where Wee1 predominates (in interphase) to one where CDK1 predominates (in M-phase). We carried out these experiments in *Xenopus* egg extracts, where Wee1A is the relevant isoform. We found that initially the interaction of Wee1A and CDK1-cyclin B1 results mainly in inactivation of CDK1, but a small amount of Wee1A phosphorylation also occurs. This Wee1A phosphorylation promotes the interaction of CDK1 with Wee1A, which in turn promotes more Wee1A phosphorylation. This provides a simple, modular mechanism for the cooperative phosphorylation of Wee1A.

SPATIAL AND TEMPORAL INFORMATION CODING BY THE NF-KB SYSTEM

Mike White
School of Biological Science, University of Liverpool, Crown St, Liverpool, L69 7ZB.

The Nuclear Factor kappa B (NF-κB) transcription factor regulates cellular stress responses and the immune response to infection. NF-κB activation results in oscillations in nuclear NF-κB abundance [1]. To define the function of these oscillations, we treated cells with repeated short pulses of tumor necrosis factor alpha (TNFα) at various intervals to mimic pulsatile inflammatory signals. At all pulse intervals analyzed, we observed synchronous cycles of NF-κB nuclear translocation. Lower frequency stimulations gave repeated full-amplitude translocations, whereas higher frequency pulses, gave reduced translocation, indicating a failure to reset. Deterministic and stochastic mathematical models predicted how negative feedback loops regulate both the resetting of the system and cellular heterogeneity. Altering the stimulation intervals gave different patterns of NF-κB-dependent gene expression, supporting a functional role for oscillation frequency [2]. I will discuss the mechanisms that lead to oscillations and cell to cell variation and the possible functions of these processes in cells and tissues.

The NF-κB system is just one of a number of biological cycles that have been discovered. Other examples include calcium signalling, transcription cycles, p53, the segmentation clock, the circadian clock, the cell cycle and seasonal rhythms. Are such cycles are a fundamental theme in the integration of biological systems? This could provide a mechanism to explain the robustness of cellular decision making processes.

References

1. D.E. Nelson, A.E.C. Ihekwaba, M. Elliott, J. Johnson, C.A. Gibney, B.E. Foreman, G. Nelson, V. See, C.A. Horton, D.G. Spiller, S.W. Edwards, H.P. McDowell, J.F. Unitt, E. Sullivan, R. Grimley, N. Benson, D. Broomhead, D.B. Kell & M.R.H. White. (2004) Oscillations in NF-kB signaling control the dynamics of gene expression. *Science* **306**: 704-8.

2. L. Ashall, C.A. Horton, D.E. Nelson, P. Paszek, C.V. Harper, K. Sillitoe, S. Ryan, D.G. Spiller, J.F. Unitt, D.S. Broomhead, D.B. Kell, D.A. Rand, V. Sée, and M.R.H. White. (2009) Pulsatile stimulation determines timing and specificity of NF-kappa B-dependent transcription. *Science*, **324**: 242-246.

DYNAMICS AND VARIABILITY IN ERK2 RESPONSE IN INDIVIDUAL LIVING HUMAN CELLS

Cellina Cohen-Saidon, Ariel A. Cohen, Alex Sigal, Yuvalal Liron and Uri Alon

Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, ISRAEL

Background: Cells respond to external signals by means of signal transduction cascades. Signaling culminates in translocation of regulatory proteins to the nucleus where they control gene expression. Signal transduction cascades are usually studied on cell averages, masking variability between individual cells. Such signaling systems need to function in the face of large cell-to-cell variation in protein concentrations or cell size.
Objectives: Here we ask how is the response of signaling systems affected by these cell-to-cell variations. Are there aspects of the response which are more robust to cell-to-cell variations than other aspects?
Methods: We studied the dynamical response of ERK2, a classically studied MAPK signaling protein, by means of fluorescent tagging at the endogenous chromosomal locus and under native regulation in individual living human cells. We monitored the ERK2 nuclear accumulation by time-lapse microscopy upon cell stimulation with specific growth factor.
Results: We find that cells show wide basal variation in ERK2 nuclear localization. After signaling, cells show a fold-change response, where nuclear accumulation is proportional to each cells basal level. Nuclear levels then decline and show exact adaptation to the original basal level of each cell. The timing of the ERK2 response is more precise between cells than the amplitude.
Conclusion: The present work of ERK2 dynamics in individual cells shows that despite large variations in basal levels, the system shows a fold-change response, exact adaptation and precise timing of the response. The fold-change response mechanism and the exact adaptation can be explained by one of the most common network motifs in topological networks, the incoherent feedforward loop (I1-FFL), in which an activator regulates both a protein and an inhibitor of that protein. The fold-response feature of the I1-FFL applies to the entire shape of the response, including its amplitude and duration. Fold-response can help buffer cell-to-cell variation in the level of regulatory proteins, and help overcome noise in the signal.This provides a view of this signaling pathway at the individual cell level and suggests that fold rather than absolute changes in nuclear level characterize the response of this pathway.

MOLECULAR BIOMARKERS FOR INDIVIDUAL DIFFERENCES IN HUMAN SIGNALING CASCADES

Ludmila Cuninkova, Ermanno Moriggi, and Steven A. Brown.
Institute of Pharmacology and Toxicology, University of Zurich, Switzerland

Intracellular communication is a key aspect of each biological system and is mediated by signal transduction cascades. Because these transduction cascades are conserved in most cell types, we developed a lentivirus – based reporter system that allows us to measure expression profiles for chosen cellular pathways directly in human primary fibroblast cells. Our goal is to look at the inter-individual differences in the expression pattern of major signal transduction pathways including the MAP- Kinase, NFkB, NFAT, and SRF pathways, as well as the circadian signaling pathway that regulates human daily behavior.

In our initial studies, we find that pharmacological responses to multiple pathway agonists display trait-like behavior: they vary considerably among cells taken from different individuals. In the case of the circadian pathway, these differences correlate directly with subject behavior. For example, individuals with shorter cellular circadian periods display earlier daily behavior, both under controlled conditions and in ordinary life.

Using this system, we plan to determine if selected cell signaling pathway responses can be used as qu antitative traits in linkage and association studies. If so, we'll try to identify genetic modifier loci that might correlate with a variety of difficult-to-access behavioral phenotypes that might include daily behavior, mood, and memory, whose operation depend upon these pathways. In addition, our reporters could serve as valuable biomarkers for prediction of patient response to many classes of drugs.

NUCLEAR RECEPTORS AS CONTROLLING FACTORS IN CHEMICAL METABOLISM: DETERMINATION OF INTERACTION NETWORKS CRUCIAL FOR CO-ORDINATING CELLULAR RESPONSE TO CHEMICALS

Nick Plant[1], Alexey Kolodkin[2], Frank Bruggeman[2]
1. Centre for Toxicology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK. N.Plant@Surrey.ac.uk
2. Regulatory Networks Group, Netherlands Institute for Systems Biology, Life Sciences, CWI, Amsterdam, The Netherlands.

The body is exposed to a wide range of external chemicals, both deliberately (e.g. medicines) and accidently (e.g. environmental contaminants). In addition, the body contains a large number of endogenous chemicals whose levels alter around a physiological mean: This may be as a result of circadian rhythms, normo- or pathophysiological processes. The body responds to changes in these chemical levels by altering flux through metabolic pathways, ensuring healthy physiology. Proteins involved in this biological response include active transport pumps, plus Phase I and Phase II metabolic enzymes, which together act to reduce the level of stimulating chemical through active efflux and metabolism respectively. In addition, members of the nuclear receptor super-family of ligand-activated transcription factors act as the signal capture/interpretation system, regulating expression of the previously detailed proteins to produce the optimal response to chemical challenge. This dual role between endogenous and exogenous metabolism raises a potential conflict between chemical exposure and biological functioning. It is thus vital that nuclear receptors not only capture/interpret chemical flux signals, but also co-ordinate the response to allow sensitivity to challenge by external chemicals, yet maintain robustness in endogenous metabolism. We have developed a deterministic model of the nuclear receptor interaction network that regulates expression of target proteins involved in the response to both external chemical challenge and internal steroid metabolism based upon in vitro and in vivo derived data. We have for the first time identified a canonical model of interaction between PXR and GR, PR, PPARα, AR and FXR nuclear receptors, comprising feed forward, feed backward and autoregulatory loops. Taken together these networks allows the rapid response to external chemical challenge whilst maintaining normophysiology. Moreover, this network also allows robustness for endogenous pathways, helping to prevent over-stimulation and pathophysiology.

ROLE OF mRNA GESTATION AND SENESCENCE IN NOISE REDUCTION DURING THE CELL CYCLE

Attila Csikász-Nagy, The Microsoft Research – University of Trento, Centre for Computational and Systems Biology
Ivan Mura, The Microsoft Research – University of Trento, Centre for Computational and Systems Biology

Recent innovations in experimental techniques on single molecule detection resulted in advances in the quantification of molecular noise in several systems, and provide suitable data for defining stochastic computational models of biological processes. Some of the latest stochastic models of cell cycle regulation analyzed the effect of noise on cell cycle variability. In their study, Kar et al. (Proc Natl Acad Sci U S A (2009) 106: 6471-6) found that the observed variances of cell cycle time and cell division size distributions cannot be matched with the measured long half-lives of mRNAs. Here, we investigate through stochastic modeling and simulation how the noise created by the transcription and degradation processes of a key cell cycle controller mRNA affect the statistics of cell cycle time and cell size at division. Our model consists of an encoding of the model of Kar et al. into BlenX, a stochastic modeling formalism supporting the extensions necessary to represent multiple synthesis (gestation) and degradation (senescence) steps in the regulation of mRNAs. We found that few steps of gestation and senescence of mRNA are enough to give a good match for both the measured half-lives and variability of cell cycle-statistics. This result suggests that the complex process of transcription can be more accurately approximated by multi-step linear processes.

TOWARD A CONSISTENT SET OF INTEROPERABLE STANDARDS TO REPRESENT MODELS AND SIMULATIONS

Nicolas Le Novère, EMBL-EBI

A decade ago, the creation of the Systems Biology Markup Language (SBML) changed the way people exchanged, verified and re-used models in systems biology. The robustness and versatility of this standard format, coupled to a wide software support fostered the emergence of a whole area of research centred on model processing such as encoding, annotation, merging, comparison and integration with other datasets.

Other community standards of representation were created in computational systems biology, and a vertical organisation is emerging, with different layers of descriptions. While the model structure is covered by SBML and similar efforts, the biological semantics can be described using BioPAX while models and pathways can be graphically represented using the languages of hte Systems Biology Graphical Notation (SBGN). Computing glues permit to related the components of the different layers. For instance one can generate SBGN maps from SBML using the Systems Biology Ontology (SBO) and convert SBML into BioPAX using the controlled MIRIAM annotations.

Independently to the systems biology community, other scientists developed their own standards of representations, such as CellML (physiology), NeuroML (neurophysiology), SimileXML (ecology), with more on the way. More recently, new languages appeared that complement the model descriptions, such as SED-ML to describe the simulation experiments or SBRML to encode the numerical results. The developers of all those initiatives are now in contact, and try to improve the interoperability of the languages.

One can hope to see, in a not too elusive future, the creation of a coherent set of non-overlapping standards, similar to the W3C standards for the World Wide Web, that will cover the various modelling approaches and scales, but also the different levels of discourse and representation in Systems Biology.

STEPS: REACTION-DIFFUSION AND MEMBRANE POTENTIAL SIMULATION IN COMPLEX 3D GEOMETRIES

Iain Hepburn[1], Weiliang Chen[1], Erik De Schutter[1,2]
[1] Computational Neuroscience Unit, O.I.S.T, Okinawa, 904-0411 Japan
[2] Theoretical Neurobiology, University of Antwerp, B-2610 Antwerpen, Belgium

STEPS (STochastic Engine for Pathway Simulation) is a platform for simulation of signalling pathways where the spatial organization and morphology of the cell can be represented by complex 3D tetrahedral meshes[1]. Our core algorithm is based on Gillespie's Direct Method extended for membrane reactions and diffusive fluxes between tetrahedral voxels.

STEPS supports a growing number of open-source and research-licensed mesh-generation packages. These packages can be used to create high-quality meshes representing the required morphology with a variety of techniques that include encoding algorithms to build a model from parameterized data and reconstructing from constructive solid geometry or boundary representation input.

A recent addition to our software is the EField object, which simulates electrical potential and currents in the 3D mesh and is coupled to the reaction-diffusion computation. A method was devised which computes the effect of membrane currents on membrane potential and spread of currents throughout nodes in the tetrahedral mesh. Voltage-gated Ion channels may be represented by their conducting states with Markov-transitions between states governed by voltage-dependent rate equations. This allows for complete simulation of voltage-gated channels in the cell membrane along with the intracellular signalling pathways.

The user-interface is in Python, a powerful and versatile scripting language that is easy to learn, though the internal computations are carried out in C++ for greater efficiency. STEPS runs on various platforms, including Unix, Mac OSX and Windows.

References:

[1] Wils S, De Schutter E: STEPS: modeling and simulating complex reaction-diffusion systems with Python. Front Neuroinf 2009, 3:15

LOGIC MODELING OF A NORMAL AND TRANSFORMED LIVER AS A MEANS TO LINK PATHWAY MAPS TO CELL SIGNALING DATA

Julio Saez-Rodriguez[1,2,3], Leonidas Alexopoulos[1,2,3], Jonathan Epperlein[1,2], Regina Samaga[4], Steffen Klamt[4] , Douglas A. Lauffenburger[1,3] , and Peter K. Sorger [1,2,3]

[1] Center for Cell Decision Processes [2] Harvard Medical School, Department of Systems Biology, Boston, MA 02115,USA [3] Massachusetts Institute of Technology, Department of Biological Engineering, Cambridge, MA 02139, USA [4] Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, D-39106, Germany

Pathway maps are useful abstractions of signaling networks but have two key limitations: they are not computable models that can be compared to functional data, and they are not cell-specific, a significant limitation because it is precisely biochemical differences between normal and diseased cells that are targeted for pharmaceutical intervention. We have recently developed an efficient method to construct predictive logic models of signaling networks based on generic pathway maps and high-throughput functional data (Saez-Rodriguez et al., *Mol. Sys. Biol*., in press). The method is embedded in the toolbox *CellNetOptimizer* that works in concert with *DataRail* (Saez-Rodriguez et al, *Bioinformatics*, 2008), a complementary toolbox for managing and transforming varied data.
In this contribution, we use this approach to create cell-specific models of signal transduction of primary hepatocytes and four cell lines of hepatocellular carcinoma (HCC). Comparison of the five resulting models reveals that, while all cell types have different wiring, the differences between primary hepatocytes and HCC cell lines are more significant, and can be ascribed to the rewiring of three key pathways that confer transformed hepatocytes oncogenic properties. Furthermore, the network optimization itself provided insight into the signaling networks, since regions of systematic failure of data fitting pinpointed side effects of drugs.

MULTISCALE MODELLING IN SYSTEMS BIOLOGY

Alexander Fletcher[1,2], James Osborne[2,3], David Gavaghan[2,3], Philip Maini[1,2]
[1]Mathematical Institute, University of Oxford, United Kingdom
[2]Oxford Centre for Integrative Systems Biology, University of Oxford, United Kingdom
[3]Computing Laboratory, University of Oxford, United Kingdom

Problems in systems biology are intrinsically multi-scale, with processes occurring on many disparate spatial and temporal scales. We present a multi-scale framework for computational modelling in systems biology. Utilising the natural structural unit of the cell, the framework consists of three main scales: the tissue level (macro-scale); the cell level (meso-scale); and the sub-cellular level (micro-scale). Cells are modelled as discrete interacting entities using either an off-lattice tessellation, or a vertex-based model. The behaviour at the tissue level is currently represented by field equations for nutrient or messenger diffusion, with cells functioning as sinks and sources. However, the versatility of the framework facilitates the implementation of more biologically realistic models, for example dynamic vascular networks. The sub-cellular level concerns numerous metabolic processes and models interaction networks and signalling pathways by ordinary differential equations or rule-based models. The modular approach of the framework enables much more complicated sub-cellular behaviour to be considered. Interactions may occur between all spatial scales.

The multi-scale framework is implemented in an open source software library called Chaste (http://web.comlab.ox.ac.uk/chaste). This software library is written in object-oriented C++ and developed using an agile approach. All software is tested, robust, reliable and extensible. We introduce the Chaste computational framework and discuss both its functionality and development. This framework is illustrated by presenting applications in developmental biology, tumour growth and bacterial biofilm formation.

BIOCHARTS: MULTISCALE MODELING OF COMPLEX BIOLOGICAL SYSTEMS

Hillel Kugler[1], Antti Larjo[2] and David Harel[3]

1) Microsoft Research Cambridge; 2) Tampere University of Technology;
3) The Weizmann Institute of Science

We address one of the central issues in devising tools for the modeling and analysis of biological systems, that of linking high-level (e.g., inter-cellular) information with lower level (e.g., intra-cellular) information. Without resolving this issue, it will be impossible to produce useful models for truly complex systems, such as complete multi-cellular organs or organisms. We propose a two-tier compound visual language, Biocharts, that links the two levels, and which is geared towards building fully executable models of biological systems. We illustrate the language and associated modeling approach by developing a model of bacterial chemotaxis and metabolism, emphasizing the ability to integrate the different levels of the system's behavior and execute the model as a whole.

DATA ANALYSIS AND PARAMETER ESTIMATION OF FRAP EXPERIMENTS USING VIRTUALFRAP

Li Ye, Ann E. Cowan and James C. Schaff

Center for Cell Analysis and Modelling, University of Connecticut Health Center, Farmington, CT 06030, USA

Rapid advances in fluorescence probe and imaging technologies now provide easily accessible tools for biologists to perform highly detailed analysis of molecular interactions in living cells. However it can be difficult to extract accurate parameters from these experiments because of the complex interplay of diffusion-reaction events with the morphology of the cell. As a result, only a small fraction of the available spatiotemporal information is utilized, and in many cases analysis remains at a qualitative level. VirtualFRAP (VFRAP; http://vcell.org/vfrap) is data centric tool for quantitative analysis of FRAP (Fluorescence Loss After Photobleaching) experiments that estimates diffusion rates, mobile fractions, and binding kinetics by comparing the entire spatiotemporal experimental data with predicted fluorescence using reaction-diffusion equations solved on the particular cell geometry. It is assumed that the participants will have a basic understanding of quantitative aspects of fluorescent microscopy. Multiple examples will be presented to illustrate the process of model discrimination and parameter estimation including the limiting cases of fast binding kinetics and fast diffusion.

LINKING MODELS TO DATA VIA *SB-PIPELINE*

J. Saez-Rodriguez*, J. Muhlich*, A. Goldsipe, J. Epperlein, L. G. Alexopoulos, B. Millard, B. Jouguin, J. Burke, C. Lopez, R. Samaga, S. Klamt, D. A. Lauffenburger, P. K. Sorger
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• Presenting authors

The process of constructing and testing models, particularly those that incorporate significant prior knowledge, involves multiple steps that are currently very poorly integrated. We have developed *SB-Pipeline* to create an effective workflow based on public standards and modern software practice. *SB-Pipeline* is a multi-faceted software platform that pulls together all of the steps involved in collecting and transforming primary data; constructing, annotating and calibrating models; and distributing and sharing simulations and analyses. *SB-Pipeline* is primarily concerned with data and model management for the purpose of calibration, and implements a robust system for tracking the provenance of data, links between data and models, and the origins of model assumptions in data or the literature. *SB-Pipeline* is a collection of discrete but interoperable software tools, rather than a single integrated system, and incorporates standard protocols for import and export of data. In this tutorial we will present three modules of *SB-Pipeline: DataRail*, *CellNetOptimizer* and *SB-Wiki*, and use a data-set of high-throughput functional data of signal transduction in liver cells (Alexopoulos et al., *submitted*) to illustrate its use.

- *DataRail* is an open source MATLAB toolbox for managing, transforming, visualizing, and modeling data, in particular the varied high-throughput data encountered in Systems Biology (Saez-Rodriguez et al., *Bioinformatics*, 2008). It supports data-driven models, in particular Multiple Linear Regression (MLR) and Partial Least Squares Regression (PLSR).
- *CellNetOptimizer* (CNO) is a MATLAB toolbox to turning pathway maps into logical models that can be calibrated against experimental data (Saez-Rodriguez et al., *Mol. Syst. Biol. in press*), generating functional, predictive, cell-type specific models of mammalian signal transduction.
- *SB-Wiki* is a wiki-based system which utilizes Semantic Web technologies and a lightweight data entry and cataloging framework to support collaborative management of unstructured and semi-structured Systems Biology data. *SB-Wiki* also tracks the experimental data used to train or evaluate models, such as details of the biological setting (species, cell type, growth or culture conditions) and experimental protocol.

SB-Pipeline resources can be downloaded from http://code.google.com/p/sbpipeline/ MATLAB is required to run *DataRail* and *CellNetOptimizer*.

References:

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2. Saez-Rodriguez, J., Alexopoulos, L.G., Epperlein, J., Samaga, R., Lauffenburger, D. A., Klamt, S., and Sorger, P. K. (2009). Discrete logic modeling to link pathway maps and functional analysis of mammalian signal transduction, *Mol. Syst. Biol. in press*, doi:10.1038/msb.2009.87.
3. Alexopoulos, L, Saez-Rodriguez, J., Cosgrove, B., Lauffenburger, D.A., Sorger, P. K. Networks reconstructed from cell response data reveal profound differences in signaling by Toll-like receptors and NF-κB in normal and transformed human hepatocytes, *submitted*.

TUTORIAL ON SYSTEMS BIOLOGY LINKER (SYBIL) – A TOOL FOR ANALYSIS OF BIOPAX PATHWAY DATA AND CONVERTING IT TO SBML FORMAT

Oliver Ruebenacker, Ion I. Moraru, and Michael L. Blinov
Center for Cell Analysis and Modelling, University of Connecticut Health Center, Farmington, CT 06030, USA

We describe a framework for using BioPAX data to create computational models that we are developing as a part of the Virtual Cell (http://vcell.org) modeling and simulation environment. Systems Biology Linker (SyBiL, http://vcell.org/biopax) is a tool for querying, analyzing and visualizing BioPAX data, and converting BioPAX to SBML. Based on the Jena Semantic Web Framework for Java, Sybil supports handling of generic RDF/OWL data as well as functions specific to handling SBML and BioPAX data. SyBiL uses an OWL-based bridge format called SBPAX as part of a generic approach to integrate model-centric formats similar to SBML with pathway-centric formats similar to BioPAX. SyBiL offers various visualization modes showing reaction networks to varying degrees of details, including displaying nodes for reactions only, displaying Petri nets consisting of nodes representing reactions, reactants, products and catalysts, and displaying composition of complexes.

THE ROLE OF NO-PKG IN THE INDUCTION OF CEREBELLAR LTD

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Long-term depression (LTD) is a persistent reduction in the efficacy of the synaptic transmission that results from the removal of AMPA receptors (AMPARs) from the postsynaptic membrane. In Purkinje cells, LTD can be induced by increasing the postsynaptic calcium concentration ($[Ca^{2+}]$) (*Neuron*, 2007, vol. 54: 787), which indicates that no other signal produced by synaptic activity is required for the occurrence of LTD. However, recent data showed that a nitric oxide (NO)-cGMP-dependent protein kinase (PKG) pathway can act upstream of Ca^{2+}-signals to modulate Ca^{2+}-induced LTD. To gain insights on the action of NO-PKG, we built a computational model of the signaling pathways involved in cerebellar LTD induction.

The model was implemented stochastically with the program STEPS. The model consists of a well-mixed biochemical network containing the principal pathways involved in the (NO-PKG, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK)). In our model, PKC phosphorylates AMPARs, leading to their removal from the postsynaptic membranes, while protein phosphatase 2 A (PP2A) dephosphorylates the receptors. Furthermore, PKG phophorylates G substrate promoting its binding to PP2A, inhibiting its activity.

The first stage of our work consisted of simulating the LTD induced by $[Ca^{2+}]$. Our results show that there is a sigmoidal relationship between the magnitude of LTD and the amplitude of the postsynaptic $[Ca^{2+}]$, as has been demonstrated previously (*Neuron*, 2007, vol. 54: 787). Furthermore, we concluded that cerebellar LTD can be induced only by increasing postsynaptic $[Ca^{2+}]$ as long as $[Ca^{2+}]$ surpass a threshold value.

To verify the role of NO-PKG pathway in this process, we simulated the presence of a NO donor while elevating the postsynaptic $[Ca^{2+}]$. Our results indicate that NO-PKG pathway decrease the half-maximum $[Ca^{2+}]$ required to induce LTD, without altering the magnitude of LTD observed. These results point out that, in Purkinje neurons, the combined action of PKC and PP2A on AMPARs are responsible for the half-maximum $[Ca^{2+}]$ necessary for the induction of LTD. However, the maximum depression observed is a result not only of the action of these two enzymes, but also is determined by the trafficking of AMPARs between synaptic and extra-synaptic membranes.

Acknowledgements: We thank Keiko Tanaka and George Augustine for sharing their data.

BIOLEGO – A PLATFORM FOR DYNAMIC SYSTEMS MODELING

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BioLego an interactive modeling and simulation platform developed by Strand Life Sciences provides a user-friendly interface to create dynamic models of biological systems. The ability to visualize a mathematical model as an intuitive and information-rich network of biological interactions is a prominent feature of BioLego. Complex biological networks can be also be visually simplified in BioLego to enhance biological understanding while retaining the underlying interconnectedness. The model building work flow is interactive and each model can be annotated in detail. All the model components can be easily searched, accessed and viewed from the interface. BioLego can also handle a large number of literature references with direct links to PubMed thus contributing to the building of an exhaustive and model specific knowledge-base.

Experiments or simulations in BioLego are based on creation and use of Parameters – Values pairs that capture perturbations to the modeled system under different conditions enabling the design of experiments that simulate disease evolution, clinical protocols and therapeutic regimens. Simulation results can be viewed using a suite of dynamically linked-visualizations.

Increased understanding of the model behavior, hypothesis generation and testing is supported in BioLego by the ability to

- run different kinds of dynamic and steady state analyses the model
- link multiple experiments
- monitor events of interest during the course of a simulation and
- switch off parts of a network to run simulations on sub networks of interest

BioLego supports user management of large numbers of experiments with the ability to store, quickly retrieve, visualize and compare results. Computationally intensive simulations can be run asynchronously in the background. Projects and data can be easily shared across multiple users enabling modular development of large models. BioLego is powered by a set of robust DAE solvers to simulate biological systems with interactions of varying time-scales.

MOLECULAR INTEGRATION OF CELLULAR METABOLISM AND MEMBRANE EXCITABILITY IN CEREBELLAR PURKINJE NEURONS

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Calcium homeostasis and membrane excitability are crucial to normal function of cerebellar Purkinje neurons. Normal Purkinje cell physiology involves phosphoinositide turnover and calcium signaling. Excitability in the Purkinje cell (PC) membrane involves calcium influx through voltage-gated calcium channels, as well as potassium efflux through (i) voltage-gated, and (ii) calcium-activated voltage-gated channels (primarily the BK channel). The interaction between phosphoinositide-induced calcium signaling and calcium-activated/voltage-gated potassium channels have not been explored extensively. Our objective was to use this subsystem in a computational model to explore the integration of cellular metabolism and membrane excitability in cerebellar PCs. We used parallel models in NEURON and Virtual Cell (VCell), two simulation platforms, to guide reproduction of a physiologically relevant model of cerebellar PC electrophysiology in VCell. We then combined our published calcium metabolism model (created in VCell) with the electrophysiological model. In this comprehensive model, we investigated the influence of IP3R1-mediated calcium release from smooth endoplasmic reticulum (SER) close to the plasma membrane (PM) on the activity of the BK channel and thus on membrane potential. The model predicts that, in the cerebellar Purkinje neuron spine, supralinear IP3R1-mediated calcium release into a submembrane shell can activate BK channels. When coupled with synaptic conductance changes, this activation of the BK channels increases the rate of repolarization (RR) of the spine. As the voltage changes in the spine propagate to the soma, the corresponding RR in the soma is also increased. Simulation of IP3R1 ko abolishes any increase in the RR in both the spine and the soma. Reduced abundance of IP3R1 (as found in some mice and humans with cerebellar ataxia; in model, 10% - 50% of the original value), almost completely abolishes any increase in the RR, in both spine and soma. Increasing the sensitivity of IP3R1 to IP3 restores normal IP3R1-mediated calcium, and restores increased activation of the BK channels. The resulting RR of both the spine and the soma are also restored. These results suggest that the BK channel may play a role in integrating signals from cellular metabolism and membrane excitability.

APOPTOSIS OR NECROSIS OR SURVIVAL: MATHEMATICAL MODELLING OF CELL-FATE DECISION IN RESPONSE TO DEATH RECEPTOR ENGAGEMENT

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Cytokines such as TNF and FasL can either trigger death or survival depending on the cell lines and cellular conditions. The mechanistic details of how a cell chooses among these cell fates are still unclear. The understanding of these processes is important since they are altered in many diseases, including cancer and AIDS.
Using a discrete modelling formalism, we present a mathematical model of cell fate decision recapitulating and integrating the most consistent facts extracted from the literature. This model provides a generic high-level view of the interplays between NFκB pro-survival pathway, RIP1-dependent necrosis, as well as the apoptosis pathway in response to death receptor-mediated signals. Wild type simulations demonstrate robust segregation of cellular responses to receptor engagement. The model also reproduces available qualitative information on the key protein knockdown phenotypes and enables the prediction of the effects of novel knockdowns. *In silico* experiments simulate the effect of ligand removal at different stages of the pathways suggesting experimental approaches to further validate and specialize the model for particular cell types. We further propose a reduced conceptual model implementing the logic of the decision process. This analysis gives specific predictions regarding cross-talks between the three pathways as well as the transient role of RIP1 protein in necrosis, and confirms the phenotypes of new mutations. Our wild type and mutant simulations provide novel insights to restore apoptosis in defective cells.
The model is a first step towards understanding how the decision between three outcomes is made. Moreover the model can be challenged in order to test and invalidate contradictory or controversial data from the literature. Finally the model also constitutes a valuable reasoning tool that can propose experiments for novel biological insights.

VIRTUAL BIOBRICKS

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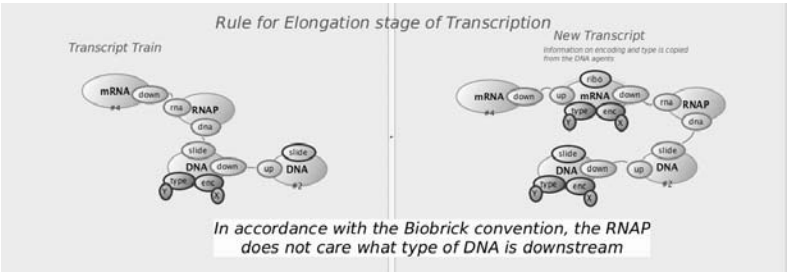
Much of the future of synthetic biology depends on an engineering approach being taken, based on standardised reusable parts which can be composed in a modular fashion to create complex systems. To this end the Biobrick Registry was created. The registry is made up of Biobrick parts which will work together in a predictable manner (see Nature Biotechnology 26 2008. Refinement and standardization of synthetic biological parts and devices. Canton B, Lano A, Endy D.). Biobrick parts are organised according to type, with each type having characterisable properties. What is missing from this engineering approach is a formal language that can capture the complex behaviour of the systems in a way that is as modular, detailed and reusable as the parts themselves. Such a language would allow detailed and transparent modelling and offer the means for accurate prediction of how various synthetic constructs would behave. Rule-based languages are a natural choice for this due to their flexibility and concision in expressing situations of combinatorial complexity such as various occupancy states of promoters. Indeed Ty Thompson has recently proposed a rule-based methodology to model the mechanics of transcription and translation (see Rule Based Modeling of BioBrick Parts). One might think of Thomson's method as the rule-based analog of a recent work based on the usage of ODEs (see eg Curr Opin Biotechnol. 2009 Aug;20(4):479-85. Computational design tools for synthetic biology. Marchisio MA, Stelling J).
We wish to present an extension of this framework to make the rules reusable and organised in an appropriate manner. Specifically, we use hierarchies to organise agents into classes (as in Transactions on Computational Systems Biology XI 2009. Rule Based Modelling and Model Perturbation. Danos V, Feret J, Fontana W, Harmer R, Krivine J.). This enables new parts to be classified using a schema similar to the Biobrick Registry. We also add `colours' to the internal states of sites which means that rules can be instantiated to many instances of different parts and do not need to be rewritten multiple times within a model. An example of such a rule is shown below. Rules that are specific to Biobrick parts could be incorporated into the parts data sheet on the registry and added to the modelling framework when the part is being used. We believe that the resulting framework which is being implemented would be a versatile tool enabling users to model complex devices with a minimum of modelling experience.



BINDING DETERMINANTS OF INTERACTIONS BETWEEN ANTIAPOPTOTIC BCL-2 FAMILY PROTEINS AND APOGOSSYPOLONE.

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Small-molecule compounds that induce apoptosis in tumor cells by inhibition of antiapoptotic Bcl-2 family proteins hold significant promise for anticancer therapies. Apogossypolone (ApoG2) is a derivative of the small-molecule anticancer drug gossypol. The modification involved the removal of two reactive aldehyde groups on the gossypol polyphenolic rings. Recent experimental studies showed that ApoG2 is a potent inhibitor of the antiapoptotic proteins such as Bcl-2, Mcl-1 and Bcl-xL (K_i values of 35 nM, 25 nM and 660 nM, respectively) resulting in its high antitumor activity. The structural basis of a strong binding of ApoG2 to antiapoptotic proteins remains unclear, whereas its characterizing may be beneficial for further improvement of anticancer drugs. One of the most efficient ways to elucidate the structural basis of the binding affinities of ligands with proteins is via the use of computational docking which is capable to achieve a level of details that is not accessible to experiment. The main problem with the use of modern docking techniques is taking account of receptor flexibility. Here, we report the results of the use of computational docking by ROSETTALIGAND program combined with AutoDock Vina program to establish the structural basis of the binding affinities of ApoG2 with antiapoptotic proteins Bcl-2, Bcl-xL, and Mcl1. The protein-ligand docking by global optimization of the scoring function included the coarse-grained stage of global search by AutoDock Vina program followed by the stage of local refinement using ROSETTALIGAND program taking into account the receptor flexibility. Depending on the protein studied, 3-10 clustered poses of the ligand were obtained at the first stage that were then refined using ROSETTALIGAND program to select the pose with best scoring function. The results of simulations showed that stronger binding of ApoG2 as compared to gossypol is caused by greater complementarity between ApoG2 and canonical hydrophobic grooves of antiapoptotic proteins that allows the formation of extra hydrogen bonds with the most significant increase in their number for the interaction with Mcl-1.

COMPUTATIONAL MODELING OF EPIDERMAL GROWTH FACTOR RECEPTOR SIGNAL TRANSDUCTION NETWORK

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Epidermal growth factor regulates cell growth, differentiation and proliferation. Our research is to model signal transduction network using course-grained computational modeling method. As our best knowledge that our research is the first EGFR signal transduction network including all three major pathways, PI3K pathway, MAPK and PLC pathway, to be studied using course-grained modeling method. In this study, we want to understand the dynamic behavior of epidermal growth factor (EGF) receptor signal transduction network by developing mathematical models to make experimentally testable predictions, to explain unexpected experimental results, and to elucidate drug response on pharmacological experiments. These predictions are validated by experimental data.
A course-grained systems biology modeling method, Causal Mapping (CMAP) [Weinreb et al, 2006], is applied to model EGFR signal transduction network. CMAP uses difference equations formalize an analytical inference of the dynamics of the elements in the signal transduction network. Random numbers generated by computer are used as the weights to represent rate constants. Concerning the difficulty of experimental test the predictions and the constraints speed of simulation, building a simple model that can explain complex biological processes is our ultimate goal. Our focus is to investigate the EGFR signal transduction and protein-protein interactions at system level. The simulation results (ensemble) are in a close agreement with experimental data qualitatively such as shape and some sets in the ensemble are in a close agreement with experimental data quantitatively.
By comparing CMAP as a course-grained modeling method to Ordinary Differential Equation (ODE) as detailed modeling method, CMAP is easier to visualize the elements interactions in the model with graphical causal mapping, and the simulation is faster. The limitation is that some detailed information is not captured by the method.

NCK FUNCTION IN TYROSINE KINASE SIGNALING TO THE ACTIN CYTOSKELETON

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Tyrosine kinase signaling leads to the post-translational modification of proteins and their binding partners. These modifications lead to the membrane recruitment of signaling proteins, promoting an increase in their local concentration, which results in a cellular response to the phosphorylation of tyrosine residues. Nck, an SH2/SH3 adaptor protein, functions in tyrosine kinase signaling by linking tyrosine phosphorylation on the membrane with binding partners, such as N-WASp, that function in facilitating actin nucleation and polymerization. However, quantitative and mechanistic aspects of signaling through Nck remain poorly understood. To explore the linkage of Nck to the actin cytoskeleton, our lab developed a system in which Nck SH3 domains can be aggregated on the plasma membrane following antibody application. Aggregation of Nck SH3 domains results in localized actin polymerization in the form of actin comet tails. Using the *Virtual Cell*, we have built a comprehensive, quantitative actin cycle model. With this model, we have produced predicted results that have been confirmed in vivo. This model predicts experimental comet tail length, actin distribution within the comet tail, and maximum actin concentration in the tail based on the number of molecules in the aggregate and the speed at which the aggregate is moving across the cell surface. We have also adapted the model to test the implications of the recent findings that binding of two N-WASp molecules to the Arp2/3 complex enhances actin nucleation and polymerization when compared with single N-WASp activation of the Arp2/3 complex. The combination of modeling and precise experimental manipulation provides unique insights into the relationship between increased local concentration of Nck and resulting localized actin polymerization.

A BIOPHYSICAL ANALYSIS OF TRANSCRIPTION INITIATION IN BACTERIA

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Bacterial RNA polymerase (RNAP) is the central enzyme of gene expression, and initiation of transcription by RNAP is a major point in regulation of gene expression. In the first and usually rate-limiting step of transcription initiation RNAP binds to double stranded DNA (the closed complex formation) and subsequently opens the two strands of DNA (the open complex formation). While a subject of intensive experimental research efforts during the last three decades, the mechanism by which RNA polymerase forms the open complex is still unclear [1]. We recently developed the first quantitative model of the open complex formation by bacterial RNAP [2], which is based on statistical mechanics. The model shows a very good comparison with both genomics and biochemical data, with no free parameters used in model testing. The model strongly supports a two step hypothesis for the open complex formation, and allows accurately estimating kinetic parameters of transcription initiation for any sequence of interest.

As a computational application of the model, we analyze poised promoters - sequences that are characterized by high binding affinity but slow rate of transition from closed to open complex – in bacteria. Possible existence of a considerable number of poised promoters in genome has been hypothesized in literature, particularly in the context of false positives obtained in experimental and computational searches for promoters. We show that promoter poising is significantly reduced by binding specificities of (physically independent) RNAP domains that interact with -10 box single-stranded and double-stranded DNA [3]. We also show that this effect is not due to generic properties of protein-DNA interactions, and argue that RNAP is designed to reduce promoter poising in genome. However, despite this reduction, the number of poised promoters still corresponds to ~ 30% of strongly bound sequences in bacteria. This number roughly matches with the lower bound of reported false positives in RNAP ChIP-chip experiments, which suggests that poised promoters are a major contributor to false positives in searches of bacterial promoters.

[1] B Young, T Gruber, and C Gross. 2004. *Science* 303:1382-1384.
[2] M Djordjevic and R Bundschuh, *Biophysical Journal* 94: 4233 (2008).
[3] M Djordjevic, submitted (2009).

Filamin A: A MOLECULAR SHOCK ABSORBER

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Filamin A is a ubiquitously expressed cytoplasmic protein, which functions as a cross-linker, creating 3-D networks with actin filaments. Additionally, as it contains an integrin-binding site, it can link the cytoskeleton to the extracellular matrix via integrins.

Insights how the structural design of this adaptor protein enables its mechano-sensitive functions in a diverse and dynamic environment might help to comprehend its unique role during formation and maturation of adhesion sites, and how force ultimately orchestrates the dynamics of assembly and disintegration of the actin network.

Our computational studies reveal, how filamin acts as a molecular shock absorber, thus protecting the actin network from disintegration during events of extensive mechanical stress. We are able to investigate, how energy is being dissipated by the need to overcome energy barriers that create the various intermediate states within the 21st Immune globulin domain of filamin, ultimately leading to the unbinding of integrins due to mechanical force.

Thus, this computational approach enables us to observe how one structural design is able to perform its role under vastly distinct circumstances like in filopodia or lamelipodia, reducing stress on the actin filament network by the breakdown of intermediate states and eventually by abolishing one anchor point within the actin-filamin-integrin system.

A MODELLING FRAMEWORK FOR UNDERSTANDING SIGNAL TRANSDUCTION UNDERLYING CHEMOREPULSIVE RESPONSES

J. Krishnan and Aiman Alam-Nazki

Chemotaxis, the directed migration of cells in response to chemical gradients is a widespread process, with very important functions. In this talk we will focus on the first step of chemotaxis, namely gradient sensing, in the context of chemorepulsion.
Based on recent experimental work, a network of interactions has been postulated to underlie chemorepulsion in Dictyostelium. This network focuses on some key phosphoinositide lipids and enzymes underlying this process, including PLC.

We take the first steps towards understanding this process by developing a mathematical modelling framework underlying this process. We develop a mechanistic modelling framework based on the postulated network, incorporating the given reactions and diffusional transport. This network includes the presence of multiple feedback effects, which makes its behaviour highly non-trivial to understand. We systematically examine the effects of the postulated feedback mechanisms as well as other possible regulation from the receptor in leading to the desired spatial profiles of key biochemical entities. Overall our approach is a first step in the unravelling of the signal transduction complexity underlying this process.

INVERSE BIFURCATION ANALYSIS OF APOPTOSIS SIGNALLING PATHWAYS

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Apoptosis is a form of cell death in which a suicide program is activated. Defective apoptosis can be linked to a variety of diseases, e.g., insufficient amount of apoptosis causes uncontrolled cell proliferation as seen in cancer. Modelling the apoptotic protein cascades of extrinsic or intrinsic signalling pathways by systems of ordinary differential equations, the usual triggering of apoptosis in a complete, all or non fashion can be explained by continuation of steady state curves. In this talk we discuss the inverse bifurcation problem of forcing the biological switch to desired behaviour. With the problem admitting several solutions, sparsity enforcing regularization can be used to determine key reaction mechanisms and potential drug targets of the signalling network. A comparison to the subset selection approach is given.

SEGMENTATION OF PLANT EMBRYONIC CELLS USING MATHEMATICAL MORPHOLOGY AND CURVATURE EVALUATION

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Research on the growth of plant embryonic cells requires calculating the change of micro-molecules such as protein, RNA and starch, and the elements, such as calcium during their different growing stages. According to these data, their growth can be quantitatively analyzed. We hope to use the three dimensional reconstruction technology of plant embryonic cells serial sections to achieve quantitative analysis. However, because of the complexity of the sections images, the segmentation problem of the serial sections images is difficult to be solved. Mathematical morphology and curvature evaluation is widely used in segmentation of vessel-like patterns. In this paper, a novel approach based on mathematical morphology and curvature evaluation for plant embryonic cells serial sections images segmentation, is introduced. Compared with the previous image segmentation algorithm, this approach observably improves both the efficiency and robustness.

DIFFUSION, TURING PATTERNS AND CELL POLARITY

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Neuronal polarization is the process by which the recently generated neuron acquires an asymmetric shape, by the sprouting of cylindrical extensions (neurites) which are the precursors of axons and dendrites. In morphological terms, polarization stages are well defined. Initially, the cell is a sphere, hence symmetric. Then, a neurite forms in one pole of the sphere, making the neuron asymmetric. Architecturally, this is the onset of neuronal polarity. Then, other bud grows in the opposite side, and later on, more neurites emerge from different parts of the sphere, until the cell is again symmetric. In neurons in vitro, the first neurite later becomes the axon. Cell polarity can be seen like a self-organised process, in which complex mechanisms are used to establish and maintain specialised domains on the cell membrane. Here, a mathematical model for two interacting surface molecules based on Turing's reaction-diffusion equations is presented. One of them plays the role of inhibitor as a regulator of endocytosis and the other one, the activator, stimulates exocytosis. Positions of the stationary maximums are correlated with places of symmetry breaking. Relationships between positive feedback for locally amplifying distributions of signalling molecules at the plasma membrane, lateral diffusion and endocytocis rate are established for the formation of Turing patterns.

GEOMETRY INFLUENCES POSITIONING AND STABILIZATION OF THE CONTRACTILE ACTIN RING IN FISSION YEAST

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The assembly of a contractile actomyosin ring and its stable positioning are essential steps in the early stages of cytokinesis, and involve a host of key molecular players, such as F-actin and type II myosin. Here we show, using fluorescence microscopy of live fission yeast cells and their spherical mutant counterparts, that geometry, viz., shape and deformability of the cell membrane, are also crucial determinants of the stability and positioning of the ring. Contractile rings that form on the spherical end caps of wild type fission yeast or on mutant spherical cells fail to stabilize and instead slip towards the poles. We show that the dynamics of ring slippage can be quantitatively described within the framework of active hydrodynamics that includes a coupling between active contractile forces and curvature. Consistent with our theory, the slipping ring stabilizes whenever it encounters a region of local negative curvature in the same mutant cell system. Our study reveals the interplay between geometry, dynamics and cell fate, which is likely to apply in a variety of cellular contexts.

A MODEL FOR DRUG INDUCED CHOLESTASIS BASED ON CELL BIOCHEMISTRY AND CONTRACTILITY

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Cholestasis is the clinical condition where the liver does not secrete bile at the expected rate, leading to increased bile salts and pigments in the hepatocytes and serum. One of the important causes of cholestasis is bile the dysregulation of the propulsion machinery around the biliary canaliculus involving actin filaments and myosin. Bile secreted by hepatocytes is propelled towards the portal triad by the coordinated "contraction" of the bile canaliculus controlled through MLCK-MLCP mechanisms. The process is mediated by vasopressin, Ca^{2+} and IP3 that permeate through gap junctions to synchronize the sequential contraction of actin ring around the canaliculus.

Bile salts secreted by the liver undergo enterohepatic circulation. The entire pool of bile salts lies primarily in the intestines and recycles about 7 times a day. Transporters on the sinusoidal side of hepatocytes help in uptake of bile salts into the cell while those on the canaliculi secrete them into bile. A small fraction of bile salts lost in this recycling is replaced by synthesis and conjugation.

Several drugs alter the function of actin-myosin and the transporters, causing cholestasis. Some drugs can directly alter the contractile apparatus while others may have complex and indirect actions, e.g. phalloidin, that thickens the actin ring around canaliculus impeding transport to the canlicular membrane.

We have developed an ODE based systems model that captures some of the canalicular mechanisms and bile salt homeostasis. The model simulates the cholestatic action of drugs that affect any of these pathways and can be used in conjunction with an in vitro assay panel to predict the cholestatic potential of new chemical entities.

MATHEMATICAL MODELLING OF WHOLE CHROMOSOME REPLICATION

Renata Retkute[1], Michelle S. Hawkins[1], Alessandro de Moura[2] and Conrad A. Nieduszynski[1]

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All chromosomes must be completely replicated prior to cell division, a requirement that demands the activation of a sufficient number of appropriately distributed DNA replication origins. Here we investigate how the activity of multiple origins on each chromosome is coordinated to ensure successful replication. We propose a mathematical model for chromosome replication where the dynamics are based upon the parameters of individual origins. We explore two approaches for mathematical modelling: first, stochastic simulations based on our model and second, using a probabilistic model which is determined by the mechanisms underlying the replication process.

Using simulation results we demonstrate that mean replication time at any given chromosome position is determined collectively by the parameters of all origins. Combining parameter estimation with extensive simulations we show that there is a range of model parameters consistent with mean replication data. Recent time-course data contain more information than mean replication time data and allowed us to use our model to uniquely estimate many origin parameters. These estimated parameters enable us to make a number of predictions that showed agreement with independent experimental data, confirming that our model has predictive power.

USING PATHWAY DATA IN BIOPAX FORMAT TO CREATE COMPUTATIONAL MODELS

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Thousands of biochemical interactions are available from public sources in the Biological Pathways Exchange (BioPAX) format. However, the current standard for exchange of simulation-ready biological models is System Biology Markup Language (SBML). This markup language is structurally and semantically different from BioPAX. Some conversion schemes exist, using annotations and based on simple one-to-one mappings between SBML and BioPAX objects, which ignores semantic differences and therefore often leads to significant loss of information or meaning. A comprehensive modeling framework capable of representing the complex relationships between SBML and BioPAX data is needed to take full advantage of existing pathway data in kinetic modeling, thus integrating these two formats by gluing them together.
Here we describe such a framework that we are developing as a part of the Virtual Cell (http://vcell.org) modeling and simulation environment. Systems Biology Linker (SyBiL, http://vcell.org/biopax) is a tool for analyzing and visualizing BioPAX data, and converting them to SBML. Based on the Jena Semantic Web framework for Java, SyBiL supports handling of generic RDF/OWL data (such as visualization and reasoning) as well as functions specific to handling SBML and BioPAX data. SyBiL uses Systems Biology Pathway eXchange, called SBPAX, as a generic approach to integrate model-centric formats similar to SBML with pathway-centric formats similar to BioPAX. SBPAX is an OWL-based schema that serves as a glue to integrate different data formats, despite semantic differences. Effectively, SBPAX provides a bridge between SBML and the Semantic Web world. SyBiL offers various visualization modes showing reaction networks to varying degrees of details, including displaying nodes for reactions only as well as displaying Petri nets consisting of reaction nodes and reaction participants and catalysts.

THE DYNAMICS OF TRANSCRIPTION INVOLVING ACTIVATION INDUCED DEGRADATION

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Activation induced degradation is a fairly common phenomenon, in which transcription factor (TF) is degraded after it has activated the transcription of the target gene. It is not known how this coupling of transcription activation and degradation affects the dynamics of transcription of the target gene(s) compared to dynamics in which transcription activation and degradation of TF are independent. A related phenomenon is activation induced disassembly, in which chaperones actively disassemble the transcription machinery from the promoter of the target genes to initiate next round of transcription. It has been proposed, but not tested; that Activation Induced Degradation/Disassembly (AID/D) increases the responsiveness of target gene transcription by continuously removing TF from promoter, so that target gene transcription closely follows TF level. Furthermore, in cases where a TF regulates more than one target genes, transcription of one gene will decrease TF levels and thus could indirectly inhibit the transcription of other target genes, leading to complex dynamics. With an active TF removal system, TF can have very high association rate without having to worry about low dissociation rate. This would be useful in reducing non-specific binding of TF. Noise characteristics of the system might also be different. Here I propose many advantages of AID/D which can be tested by modeling the dynamics of AID/D and comparing it with regular transcription. Further, *in vivo* dynamic studies using fluorescently labeled TF and target genes, would provide greater understanding and capabilities of transcription involving AID/D.

EVOLUTION DIRECTED SMALL MOLECULE COMBINATORIAL INHIBITION OF IL-1β EXPRESSION

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Objectives: Regulation of pro-inflammatory gene expression such as Interleukin-1β (IL-1β) [1, 2] involves complex interactions between signalling cascades (e.g. MAPK, NF-κB). Our aim is to use selective inhibitors of proteins within these cascades in combination and to identify those combinations that optimally modify nodes in the IL-1β expression network using an evolutionary algorithm (EA). **Methods:** Identifying unique combinations of inhibitor affecting IL-1β expression is a multi-objective optimization problem [3, 4]. These objectives *inter alia* are; inhibition of IL-1β expression and combination effects on cell viability. J774 macrophages were pre-incubated with inhibitor cocktails prior to stimulation with lipopolysaccharide (1 µg / mL) or DMSO (0.1%). Intracellular IL-1β concentration was measured in cell lysates by ELISA, these data in conjunction with measurements of cell viability were fed into the EA and a new population of combinations generated. The iterative 'wet-dry' loop continuing until optimal combinations were located.
Results: Successive generations of combinations have revealed their synergistic inhibition of LPS stimulated IL-1β expression. **Conclusions:** Poly-combinatorial mixtures of compounds have the scope to reveal appreciable coalistic activity for exquisite combinations of inhibitor. Future work will focus on the assay of inhibitor combinations via flow cytometric measurements and the integration of this data into an *in silico* model.

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GENOME SCALE KINETIC MODELLING OF SACCHAROMYCES CEREVISIAE

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Kinetic modeling is an important tool for increasing our understanding of biological systems. The kinetics of a reaction include all effectors of the rate of reaction: this can be inhibition, activation, binding constants, substrate concentration, product concentration and equilibrium constants (amongst others). In biological systems many kinetic parameters are difficult to measure. In vitro kinetic measurements often lack information on inhibitory and activation behaviour within the system because not all interactions are known. In vivo kinetic measurements are often difficult to obtain due to the complexity of the cellular environment. This leads to a dearth of information to model with. Here we present the preliminary steps towards producing a 'minimum information requirement' approach to metabolic kinetic modeling.

SCALABLE METHODS FOR MODELING THE EGFR NETWORK AND OTHER COMPLEX CELL SIGNALING SYSTEMS

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Computational modeling has proven to be an effective tool for studying cell signaling networks. However, the size and complexity of signaling networks like the EGFR system can overwhelm commonly used reaction-based modeling techniques, making it impossible to build and simulate detailed models of these systems without making unwarranted assumptions. We built a model of the EGFR system using a novel rule-based modeling framework that avoids the need to explicitly specify all possible species in the model. The framework is based on a formal, context-free semantic language called Kappa that we created to represent proteins and reactions in a compact yet intuitive manner. We can simulate this model using our stochastic and deterministic simulators that, like Kappa, avoid combinatorial complexity, allowing for the simulation of our EGFR model that could not be handled by any other currently available tools. Using this suite of tools, we have begun investigating the molecular signatures of healthy and diseased cells in the presence of therapeutics. In our initial studies with a c-MET inhibitor (SU11274), our model indicates that significant decreases in phosphorylation of ErbB receptors in the presence of the inhibitors requires interactions between c-MET and each ErbB receptor. Coupled with published data that shows large drops in EGFR and HER3 phosphorylation when treated with c-MET inhibitors, our model suggests direct interactions between c-MET and EGFR as well as between c-MET and HER3.

EDGE EXTRACTION OF CELL IMAGE

Zhaobin Wang & Yide Ma

With the development of biological imaging technology, a large number of cell images are obtained by various devices. Hence, the processing and analysis of cell image becomes very important. Extracting single cell from image is the first step of analysis and measurement. Here we propose an efficient method of edge extraction of cell image. Similar to watershed algorithm, this method can locate edges of every cell in the image by ridge extraction. Experimental result shows the proposed method is practical and efficient.

SURVIVAL OF THE ALIGNED: MODELLING THE CORTICAL MICROTUBULE ARRAY IN PLANT CELLS

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Plant cells typically elongate along a single growth axis. In order to sustain this anisotropy the cell requires spatially extended structures that encode the proper geometrical constraints. The most prominent of these structures is the so-called interphase cortical array. Its components are microtubules: long filamentous protein aggregates that exhibit an interesting intrinsic dynamics, in which they stochastically switch between periods of growth and shrinkage. In the cortical array the microtubules are attached to the inner side of the plasmamembrane, effectively creating a 2D system, in which the only motion is caused by (de)polymerization. Because of the reduced dimensionality growing microtubules can now collide with pre-existing ones, giving rise to an angle dependent scattering events, in which the the colliding microtubule can either alter its course to grow along side the other microtubule, switch to the shrinking state, or simply slip over the obstacle. We address the question whether these interactions are sufficient to explain to explain the high degree of orientational alignment found in the cortical array. To that end we will present results both from event driven stochastic simulations and a coarse-grained dynamical model.

SELF-ORGANIZED CYCLIC CONTRACTION PATTERNS IN MUSCLES

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Skeletal and heart muscle fibers consist of chains sarcomeres, the elementary force generating elements. Sarcomeres have been found to contract and relax repeatedly in the absence of calcium. Chains of sarcomeres exhibit spontaneous wave patterns. Waves nucleate at random locations and propagate some distance and eventually disappear. A minimal microscopic description of sarcomeres allows for spontaneous oscillations based on the interaction of actin, myosin and elastic elements. The microscopic description of chains of sarcomeres reveals spontaneous nucleation and annihilation of waves as reported in the experiments. The cyclic dynamic is excitable by perturbations of the resting state. Phenomenologically treating muscle tissue as a continuous active material uncovers the central mechanisms allowing for the cyclic contraction patterns.

A MINIMAL 3-DIMENSIONAL MODEL OF AN ATRIAL MYOCYTE WITH A REALISTIC DISTRIBUTION OF RELEASE SITES: A COMPUTATIONAL CELL BIOLOGY APPROACH

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Here we develop a 3-dimensional model of an atrial myocyte with a cylindrical geometry. Transport of calcium within the cell is modelled by diffusion, with sarcoplasmic reticulum (SR) re-filling modelled with a linear pump. This is described mathematically with a linear second order parabolic partial differential equation (PDE). Calcium release is considered to act only at discrete points within z-planes via a calcium-induced-calcium release mechanism. We take this to be a threshold process that releases calcium from the SR at a constant rate for a fixed duration comparable to the lifetime of a calcium spark. These calcium release events are regarded as source terms in the linear PDE, triggered when cytosolic calcium exceeds threshold, which mimics calcium excitability. By solving the dynamics between release events (exploiting the linearity of the PDE) we formulate a minimal model solely in terms of behaviour at the release sites. This minimal description contains all the original biology and geometry of the full model, while being computationally inexpensive. The main overhead lies in the summation of release unit activity that contributes to the spatio-temporal evolution of the calcium concentration throughout the whole cell. In contrast to ventricular myocytes, atrial myocytes do not possess a T-tubule system and hence the issue of calcium wave propagation to the cell interior is much more important for subsequent cell contraction. Our computational model allows us to exhaustively probe the dependence of wave properties (speed, shape, path through the cell), on stimulus protocols, release strength, pump-rates, and values of the effective diffusion coefficient. Importantly we find that a diffusive gap, i.e. the absence of release sites, in the sub-sarcolemmal space (consistent with experimental findings) can lead to propagation failure of centripetal waves if the initial stimulus is too weak. Thus the model is useful for exploring the functional consequences (degree of myocyte contraction) of hormonal or electrical stimulation.

SPONTANEOUS ORGANIZATION OF CORTICAL MICROTUBULE ARRAYS IN PLANTS; MODELING FROM MOLECULAR TO CELLULAR SCALES

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Microtubules confined to the two-dimensional cortex of elongating plant cells must form a parallel yet dispersed array transverse to the elongation axis for proper cell wall expansion. Collisions between microtubules, which migrate via hybrid treadmilling, can result in plus-end entrainment ("zippering") or catastrophe. Here, we present (1) a cell-scale computational model of cortical microtubule organization and (2) a molecular-scale model for microtubule-cortex anchoring and collision-based interactions between microtubules. The first model treats interactions phenomenologically while the second addresses interactions by considering energetic competition between crosslinker binding, microtubule bending and microtubule polymerization. From the cell-scale model, we find that plus-end entrainment leads to self-organization of microtubules into parallel arrays, while collision-induced catastrophe does not. Catastrophe-inducing boundaries can tune the dominant orientation. Changes in dynamic-instability parameters, such as in mor1-1 mutants, can impede self-organization, in agreement with experiment. Increased entrainment, as seen in clasp-1 mutants, conserves self-organization, but delays its onset. The molecular-scale model predicts a higher probability of entrainment at lower collision angles and at longer unanchored lengths of plus-ends. Our models lead to several testable predictions, including the effects of reduced microtubule severing in katanin mutants and variable membrane-anchor densities in different plants, including Arabidopsis cells and Tobacco cells.

ACTIVE GROWTH OF ACTIN FILAMENTS CAN LEAD TO A NON-EXPONENTIAL LENGTH DISTRIBUTION

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Important properties of the actin cytoskeleton depend on the distribution of actin filament lengths. Here, we study the growth dynamics of actin filaments, taking into account addition and removal of monomers at both ends, the different phyophorylation states of the monomers and a stochastic dephosphorylation of monomers within the filaments [1,2]. The assembly of actin is active: While energy-rich ATP-bound actin monomers are readily integrated into filaments, the dephosphorylated ADP-actin monomers only have a low affinity for the filament and easily detach from it. In contrast to unregulated filament growth, we find that the active growth can lead to non-exponential length distributions. We show that they result from a stability gradient of monomers within a treadmilling filament. This is similar to a possible mechanism of length regulation by destabilizing proteins [3].

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MICROTUBULE ORGANIZATION IN XENOPUS MEIOTIC SPINDLES.

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Extracts prepared from the eggs of Xenopus frogs are used routinely to study the assembly of meiotic spindles found in vertebrates. In these extracts, thousands of microtubules are nucleated around the chromatin mass, and with time get organized into a bipolar spindle. A spindle is a remarkable structure, and during metaphase can remain steady for hours, even though individual microtubules have a lifetime of only a few minutes. We will discuss the open questions relating to microtubules organization in the spindle, and present our computational model of the metaphase steady state. The model includes poleward flux, and turnover of microtubules arising in part from dynamic instability, and in part from the activity of depolymerizing enzymes.

TRAFFICKING + SIGNALLING = MULTIPLE COMPARTMENTAL IDENTITIES AND DIVERSE CELLULAR FUNCTIONS.

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Transport of molecules in cells is a central part of cell biology. It couples compartmental signalling to generate many key cellular phenomena, ranging from formation of distinct molecular identities in cellular compartments to maintenance of stable connection properties in thousands of synapses on neurons. Functional motifs such as oscillators, switches, and spatial patterns are known to emerge from single-compartment signalling and reaction-diffusion systems Likewise, there are common trafficking motifs shared by such as processes as SNARE and Rab GTPase-mediated trafficking, and diffusive coupling. What happens when we put them together?

The first surprise was that almost all single-compartment signalling networks collapse down into three main categories, when looked at from the viewpoint of trafficking. A similar reduction occurred with trafficking. This leaves us with a manageable number of permutations when making cellular circuits by combining signalling and trafficking. With a relatively small set of biologically plausible assumptions, all these permutations were mathematically tractable using graphical analysis. This analysis qualitatively predicted the kinds of states and properties that the system can adopt. These ranged from multi-compartment oscillators to systems with up to four stable states. Specific examples of these states were implemented as ODE models, and confirmed using steady-state analysis as well as using time-series simulations.

An analysis of state space gave the second surprise. By varying trafficking parameters, it turned out that trafficking topology exerted a larger effect on system behaviour than signalling details. For example, very dissimilar feedback networks could adopt the same states when trafficked, and SNARE trafficking could induce almost any signalling network to generate two distinct compartmental identities.

Finally, the analysis was retrofitted onto several existing simulations, ranging from compartment formation and MAPK oscillations to synaptic plasticity. Most of these existing models fit into this framework, which provided additional insights into system function.

Overall, this study provides a unifying framework to analyze a wide range of cellular trafficking and signalling situations, and shows how diverse compartmental identities and cellular functions arise.

FORCE GENERATION AND SPATIAL REGULATION OF MICROTUBULES IN THE ANAPHASE SPINDLE FROM FISSION YEAST

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The mitotic spindle is an assembly of microtubules, motors and regulatory proteins that forms spontaneously to segregate the cells' genetic material. Although it is thought that most of the components that are critical to cell division have been identified, our understanding of this process remains incomplete. An advantage of studying anaphase is that this is perhaps the simplest stage of mitosis, and the fission yeast is also one of the simplest autonomous eukaryotic organisms. In this work we considered two complementary aspects of anaphase, the generation of force by molecular motors and the spatial regulation of microtubules. We estimate forces resisting spindle elongation in fission yeast and consider how uniform elongation speeds are maintained in the presence of stochastic fluctuations. In the second part of the work we investigate mechanisms that spatially regulate MTs to ensure stable MT overlaps, and thus spindle integrity.

EVALUATING THE ROLES OF CYTOPLASMIC AMPLIFICATION AND KINETOCHORE-MEDIATED INHIBITION IN SPINDLE ASSEMBLY CHECKPOINT DYNAMICS

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The mitotic checkpoint is the major biochemical pathway acting to ensure equal chromosome distribution during cell division. The checkpoint delays chromatid segregation (i.e. anaphase) until all chromosomes have attached to the mitotic spindle through their kinetochores. This is accomplished through the production of a "wait anaphase" signal from unattached kinetochores. The local production of this signal is stopped when stable spindle attachments are established, promoting the initiation of anaphase. This remarkable system displays three characteristics that necessitate detailed quantitative study: (1) sensitivity to a single unattached kinetochore, (2) robustness to variable component levels and (3) rapid onset of anaphase following completed spindle attachment. Here we present a computational model based on cellular measurements that simulates the spatially distinct production of anaphase inhibitors from kinetochores, their diffusion into the cytoplasm and inhibition of the anaphase-promoting machinery, and subsequent deactivation. A central species in the "wait anaphase" signal is the Mad2*-complex (also known as MCC). The kinetochore Mad2*-production capacity and the cytoplasmic Mad2*-deactivation rate were measured within living cells by fluorescence recovery (FRAP) and fluorescence correlation (FCS) methods, respectively. Proposed models of checkpoint signaling presume a balance between Mad2* production and dissolution. Using our measured parameters we find that a simple balance between production and dissolution is unable to sustain checkpoint signaling. Augmentation of the model with activities of both cytoplasmic activation and kinetochore-mediated repression of p31comet, a Mad2* dissolution factor restore kinetochore activity. We explore the synergy of these activities to show how they combine to support single kinetochore sensitivity and rapid anaphase onset. We are now undertaking biological experiments to test these predictions in living cells. Model refinements supported by systematic experimentation will permit further quantitative studies of basic mechanisms underlying mitosis and provide clues to the varied response of cells to anti-mitotic agents.

DYNAMICAL MODELING OF MICRORNA ACTION ON PROTEIN TRANSLATION

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Protein translation is a multistep process which can be represented as a cascade of biochemical reactions (initiation, ribosome assembly, elongation, etc.), the rate of which can be regulated by small non-coding microRNAs through multiple mechanisms. It remains unclear what mechanisms of microRNA action are the most dominant: moreover, many experimental reports deliver controversal messages on what is the concrete mechanism actually observed in the experiment. Nissan and Parker (Nissan and Parker, RNA, 2008) have recently demonstrated that it might be impossible to distinguish alternative biological hypotheses using the steady state data on the rate of protein synthesis. For their analysis they used two simple kinetic models of protein translation. In contrary to the study by Nissan and Parker, we show that dynamical data allow discriminating some of the mechanisms of microRNA action. We formulate a hypothesis that the effect of microRNA action is measurable and observable only if it affects the dominant system (generalization of the limiting step notion for complex networks) of the protein translation machinery. The dominant system can vary in different experimental conditions that can partially explain the existing controversy of some of the experimental data. Our analysis of the transient protein translation dynamics shows that it gives enough information to verify or reject a hypothesis about a particular molecular mechanism of microRNA action on protein translation. For multiscale systems only that action of microRNA is distinguishable which affects the parameters of dominant system (critical parameters), or changes the dominant system itself. Dominant systems generalize and further develop the old and very popular idea of limiting step. Algorithms for identifying dominant systems in multiscale kinetic models are straightforward but not trivial and depend only on the ordering of the model parameters but not on their concrete values. Asymptotic approach to kinetic models allows putting in order diverse experimental observations in complex situations when many alternative hypotheses co-exist.

MODELING IN CELLUCIDATE: TACKLING COMPLEXITY, COMMUNICATIONS AND COLLABORATION

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When building computational models of cellular processes, researchers combine mechanistic understanding of individual components into a single model. However, researchers must often manually combine many possibly disparate pieces of mechanistic understanding in order to explicitly define the list of all possible complexes that may participate in each reaction in the model. For many signaling networks, the number of possible complexes and reactions can overwhelm modeling tools and necessitate unwarranted simplifying assumptions that might bias model behavior. Additionally, such models are often comprised of long and inscrutable lists of complexes and reactions. Thus, although models are based on large numbers of facts, the models themselves are poorly positioned to communicate this information.

Cellucidate is an online software platform that enables construction and simulation of models of complex biochemical networks via an intuitive graphical interface. Here, we will demonstrate how Cellucidate can be used to build models and overcome the challenges of network and model complexity, using the model itself as a communication tool, and collaborative model construction.

Complexity: Using a rule-based reaction framework, Cellucidate offers the capability to describe and simulate models that would otherwise require researchers to explicitly specify millions of complexes and reactions. Thus, the use of this framework enables us to build models of complex systems without making unwarranted simplifying assumptions.

Communication: The granularity of rules in Cellucidate closely mirrors the granularity of our understanding and our natural language descriptions of underlying events. Together with the intuitive graphical interface of Cellucidate, this facilitates communication by making Cellucidate models easy to read and understand. Additionally, researchers can share their models through Cellucidate with the entire research community, facilitating communication and reuse of models.

Collaboration: The web-based Cellucidate interface enables multiple researchers in different locations to collaborate seamlessly on a single model. Cellucidate also facilitates indirect collaboration by enabling users to build upon and modify models that are made available by other researchers through Cellucidate.

MOLECULEWIZ: A VISUAL DESIGN TOOL FOR STOCHASTIC CELLULAR MODELS

Presentation Type: Software Demo

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We have designed a user-friendly interface for specifying 3D stochastic simulations of molecular interactions in a realistic cellular environment. Our software, MoleculeWiz, provides a GUI front end to the advanced stochastic simulator MCell3 (Kerr et al., SIAM Journal on Scientific Computing, 30(6), pp. 3126-3149, 2007). The program MCell3 provides powerful, validated functionality including reactions between molecules diffusing both in volumes and on surfaces. This level of detail for simulations requires correspondingly sophisticated model specification, including molecular reaction networks and their probabilities as well as constraints on initial distributions in a 3D cellular model. This is typically done via MCell3's MDL scripting language. MoleculeWiz is designed to facilitate initial exploration with MCell3 without the overhead of learning the MDL language. The MoleculeWiz GUI allows a user to make model specifications in an intuitive graphic environment. MoleculeWiz then writes the output to MDL for MCell3. Additionally, it provides a 3D view of the cellular model space. This allows properties such as initial reactant locations to be made via point and click with a mouse, simplifying the simulation design process and also aiding in the conceptualization of the 3D simulation conditions. MoleculeWiz is developed in a cross-platform fashion with intended use on the MacOS, Linux, and Windows.

ADVANCED MODELING, SIMULATION, AND DATA ANALYSIS USING VCELL

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Recent years have seen a proliferation of technologies that aim to gather quantitative data in live cells such as molecular concentration distributions, diffusion rates, and reaction rates. Highly specialized algorithms and software are required both for proper analysis of experimental data and for building predictive models of molecular interactions. This tutorial will cover intermediate and advanced topics related to kinetic models and simulations of intracellular reaction networks in 2D and 3D spatial geometries. The Virtual Cell (VCell; http://vcell.org/) platform will be used, which is a distributed framework for building, sharing, and publishing spatially-resolved kinetic models and simulations. It is assumed that the participants will have a basic understanding of quantitative aspects of fluorescent microscopy and of biochemical principles such as rate laws and mass action. A wide spectrum of examples will be presented that show how microscopy data can be used in conjunction to molecular biology and biochemistry data to generate mechanistic and predictive models of spatial signaling and regulation in eukaryotic cells. We will present the use of stochastic and deterministic models, as well as the use of complex protocols involving advection/flow, spatially heterogenous initial conditions, and combinations of reaction-diffusion mechanisms with electrophysiology.

MULTISCALE BIOLOGICAL MODELING IN MOOSE

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Introduction

MOOSE (Multiscale Object Oriented Simulation Environment; http://moose.ncbs.res.in) is a general biological simulator. It allows the simulation of a model that spans many levels of detail—from single molecules to neuronal networks. An intuitive scheme borrowed from GENESIS [1] lets the user see objects of various types—channels, molecules, electronics—and construct models out of them. Internally, specialized numerical engines for each level of detail perform calculations efficiently. It can take advantage of cluster hardware to simulate very large scale and detailed models.

Basic Framework

- Specialized Numerical Engines – Implementations of the Hines' solver for biophysical models of neurons and a GSL- (GNU Scientific Library-) based solver for kinetics of biochemical signaling networks are now in production mode. In terms of speed of execution, the Hines' solver matches the performance of GENESIS, whereas the kinetics' solver is 10x-50x faster than GENESIS. A Gillespie solver for stochastic chemical kinetics has been implemented.
- SigNeur – SigNeur is a set of tools to specify multiscale neuronal models comprising of biochemical signaling and cellular biophysics. The user embeds reaction networks in different parts of a cell, and specifies an appropriate solver for each part of the model (e.g.: a stochastic chemical solver for small volumes). Communication is managed seamlessly between solvers. A specific example is upregulation of AMPA receptors in a spine, which is conveyed from a chemical solver to a neuronal solver, and interpreted there as an increase in maximal synaptic conductance.
- Parallelization – A developer- and user-friendly framework has been implemented using the MPI (Message Passing Interface) standard to allow MOOSE to run in parallel on a cluster. Both developers and users can think of their problem in a serial fashion, with the underlying framework managing cross-node communication and load-balancing. MOOSE has successfully cleared a large set of unit tests on a 64-node Linux based Opteron cluster.

Interfaces

- PyMOOSE – PyMOOSE [2] is a Python-based scripting interface to MOOSE implemented using SWIG (Scalable Wrapper Interface Generator). This makes available a large universe of Python libraries which can be used for data analysis, and also allows run-time communication with other Python-aware simulators.
- SBML – The capability to read and write models of chemical signaling in the simulator-independent Systems Biology Markup Language (SBML) [3] has been implemented and tested in MOOSE.
- NeuroML – An early version of a NeuroML [4] library has been implemented to allow importing of NeuroML (Version 1, Levels 1 and 2) models. This library has in turn been used in MOOSE to import single neuron models successfully.
- Graphical interface and 3D Visualization – A graphical user interface has been implemented in PyQt, which allows the user to load and run models, and to plot simulation state variables. 3D visualization support has been added for displaying neuronal models and state variables, using OpenGL and OpenSceneGraph.

Acknowledgements

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TOWARDS AN INTEGRATED MODEL OF MORPHOGENESIS IN PLANTS

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During the development of multi-cellular organisms the regulatory networks controlling growth and patterning must somehow interfere with physical processes to generate specific shapes. How this is achieved, i.e. how molecules assemble into complex systems with a particular form is not known in any organism. We are addressing this central issue in developmental biology using the shoot apical meristem of the higher plant Arabidopsis, which initiates all the aerial organs of the plant.
As a first step, we are currently trying to link the activity of regulatory genes to specific morphogenetic events. To this end, we have developed a computational pipeline, aimed at expressing gene function in terms of quantified, geometrical changes in tissue shape. Using this tool, we are monitoring anisotropy and growth rates in gene expression domains and in mutant backgrounds.
In parallel we have started to analyse the molecular basis of shape control. Using a combination of physical, mathematical and biological approaches we have provided evidence for a model where molecular networks would impact on two separable processes. First we have identified a microtubule control of cell wall anisotropy which feeds back on local stress and strain patterns. This process seems to define particular morphogenetic events and can be uncoupled from the control of overall growth patterns, associated with the rapid outgrowth of organs at particular locations.

CELL FATE DETERMINATION IN THE *ARABIDOPSIS* FLORAL MERISTEM

J. Molenaar, S. van Mourik, WUR

In this presentation we deal with modeling of the flower formation process in \textit{Arabidopsis thaliana}, starting from the genetic level. In this model plant, flowers consist of four types of organs, that grow in four concentric whorls from the floral meristem. In each whorl, the cells attain a different identity that is determined by the concentrations of five types of proteins. The well-known ABC(DE) model describes cell fate in terms of static protein concentrations that are correlated to the organ types in each whorl. We show how the mechanisms that regulate cell differentiation in the floral meristem can be understood quantitatively. To that end we develop a continuous-time model for the gene regulatory network that regulates the protein concentrations. Recent insights from experiments suggest that not only the proteins, but also their dimers are strongly involved in the regulation. These insights are incorporated in the present model , which allows for testing of these suppositions.
We show that a model including dimer dynamics is capable of describing measured protein concentrations quite well. Furthermore, the model is validated by comparing model predictions to known mutagenesis experiments. To allow for further tests and refinements, we present predictions for mutant types, that are not studied experimentally. In the final part of this talk we deal with the role of auxin in flower formation, applying a model that describes the dynamical distribution of auxin over the floral meristem and coupling auxin concentrations to the ODE model underlying the ABC(DE) model. First results indicate that this coupling indeed yields the correct patterns on the floral meristem.

Future experimental verification of these predictions will show the value of the model.

A POLARISED POPULATION OF DYNAMIC MICROTUBULES MEDIATES HOMEOSTATIC LENGTH CONTROL IN ANIMAL CELLS

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Because physical form and function are intimately linked, mechanisms that maintain cell shape and size within in tight limits are likely to be important for a wide variety of biological processes. However, while intrinsic controls have been found to contribute to the relatively precisely defined shape of bacteria and yeast cells, mediated in part via the cytoskeleton, the extent to which individual cells from a multicellular animal control their plastic form remains unclear. Here, using micro-patterned lines to limit cell extension to one dimension, we show that animal cells spread to a characteristic steady-state length that is independent of their mass, pattern-width and lamellipodial actin. Instead this homeostatic length control depends on dynamic microtubules. In exploring the role of microtubules in this process, we find that the majority of microtubules in cells on lines align parallel to the long cell axis as the result of interactions of microtubule plus ends with the lateral cell cortex. Moreover, these protrusions lead during cell extension. Significantly, a simple, quantitative model of cellular extension driven by dynamic oriented microtubules recapitulates cell spreading dynamics, the steady-state distribution of microtubules, cell length homeostasis and predicts the effects of microtubule inhibitors on cell length. Together this experimental and theoretical analysis shows that well-understood cytoskeletal dynamics impose unexpected intrinsically-defined limits on cell geometry. It also reveals mechanistically how animal cells are able to regulate their length - a process that plays a critical role in processes as diverse as gastulation and neurite outgrowth, and which has important implications for the subsequent fate of the cell.

COARSE GRAINING AND ROBUST MORPHOGENETIC FIELD FOR DROSOPHILA DEVELOPMENT

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The development of form and pattern in animal embryos has been subjected to intensive investigation over the last century. A unifying idea in these studies was that of the ``morphogenetic field", wherein biological pattern was generated by the interplay of biologically elementary actors. A key property of a morphogenetic field is its ability to ``regulate", which in classic embryology means to correct errors stemming from perturbations or natural variation. A specific instance of this property is ``size regulation", wherein the organism compensates for variation in the size of the morphogenetic field by making changes in cell fate that insure that the proportional positions of particular cell fates are preserved.

Early mathematical insights into this problem can be found in René Thom's "Mathematical theory of morphogenesis". Thom looked for a geometric and detail independent mathematical theory, which is able to classify all possible mechanisms that generate stable form. Our approach is different. We start from models with full biochemical details and by using coarse graining we obtain abstract dynamical systems descriptions across a wide range of scales. The many-to-one mapping provided by the coarse graining gathers extensive sets of interactions into a few active modes of the developmental system. These modes, that are manifestations of the morphogenetic field, can change in time from one developmental stage to another.

The error correction properties of the morphogenetic field originate from two complementary phenomena. On one side, we have the robustness by dimension compression, which is a generalization of the law of large numbers to systems that do not necessarily have large numbers of molecules of the same species, but that have many interactions between many different species (Gorban, Radulescu 2007).This property buffers variability of the interaction strengths. On the other side, the resulting morphogenetic modes generate stability by various mechanisms such as canalization by attractors and attractive manifolds, and error correcting regulatory patterns (Manu et al 2009).

We apply our methods to early segmentation of Drosophila, which is one of the most studied developmental system. The gene circuit model (GCM) considering simplified interactions between segmentation genes explains well the experimental data. First we discuss the mapping from a transcription model to the gene circuit model (GCM). We show how the genetic variation at the level of cis-regulatory modules is buffered. As a second application, we present two approximations of the GCM. The diffusionless approximation explains the canalization properties of the gap gene system, and the interacting kink approximation explains the size regulation (Vakulenko et al 2009).

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REGULATORY MODULE FOR ROBUST TEMPORAL GENE EXPRESSION IN *DROSOPHILA* NEUROGENESIS

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Precise temporal coordination of gene expression is required in various developmental processes. In *Drosophila* neurogenesis, neural stem cells called neuroblasts sequentially express a group of genes in a definite order, which generates the diversity of cell types. How do such coordinated expression patterns are controlled at the regulatory network level? By producing all possible regulatory networks of the genes and examining their expression dynamics numerically, we identify requisite regulations and predict an unknown factor to reproduce observed expression profiles in both wild type and mutants. We then evaluate the stability of the actual *Drosophila* network for sequential expression. This network shows the highest robustness against parameter variations and gene expression fluctuations among the possible networks that reproduce the expression profiles. From these results, we propose a regulatory module composed of three kinds of regulations which is responsible for precise sequential expression.

DYNAMICS OF MIN PROTEINS IN VIVO AND IN VITRO

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In the bacterium Eschrichia coli, the Min proteins oscillate between the two cell poles. At the poles they prevent cell division, which consequently occurs in the cell center. According to several theoretical studies, these Min oscillations could result from self-organization of the two proteins MinD and MinE in the presence of a membrane and ATP. We tested these predictions in an in vitro assay of a supported lipid bilayer together with purified MinD and MinE as well as ATP. We find the proteins to organize into planar or spiral surface waves. We show that the in vitro as well as the in vivo structures can be generated by the same mechanism. In short cells we find the Min proteins to switch stochastically between two coexisting stationary states. Theoretical analysis indicates that switching is caused by fluctuations on molecular scales. As the bacterium grows, we observe a transition into the regular oscillation pattern mentioned above as well as a significant reduction of intercellular fluctuations. Together, these results strongly indicate that self-organization of a small number of different proteins is used in living cells to generate spatio-temporal structures of physiological importance.

A ROBUST AND PASSIVE CONTROL OF CHROMOSOMAL TOXIN-ANTITOXIN SYSTEMS BY THE CELLULAR LEVEL OF TRANSLATION

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Bacteria multidrug tolerance prevents antibiotics from eradicating infections and is caused by a small population of dormant bacteria called persisters. Chromosomal toxin-antitoxins can enhance multidrug tolerance, presumably by enabling a dormant state that permits bacteria to resist antibiotic treatment. Stasis is induced by the toxin once it is released from an inactive complex with the labile antitoxin. How the toxin is activated in response to adverse conditions and why it is active in a small fraction of bacteria within an isogenic population has remained elusive. RelB/RelE serve as a paradigm for toxin-antitoxins and have been studied in great detail. Active RelE accumulates in response to starvation without an apparent change in the rates of protein expression or degradation. All attempts to identify further stress-dependent regulators have remained unsuccessful. Here we resolve the paradox by combining mathematical modeling with experimentation. We show that a sophisticated mechanism based on positive and negative cooperativity enables accumulation of active RelE in response to starvation without requiring changes in the rates of protein expression or degradation. We also define the elusive role of the sophisticated transcriptional regulation of *relBE*, and we show that it mainly serves to render the network robust.

A SYSTEM TO STUDY MITOTIC ENTRY AND CHECKPOINT RECOVERY WITH HIGH SPATIAL AND TEMPORAL RESOLUTION

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Entry into mitosis is regulated by a complex signalling network, converging on activation of mitotic Cyclin-Cdk complexes. To prevent transmission of mutations, this network is strictly controlled by DNA-damage checkpoints. In fact, DNA-damage checkpoint signalling and mitotic entry signalling can both modify each other. Because of these modifications, the decision to enter mitosis is taken differently after recovery from a DNA-damage checkpoint compared to during unperturbed growth.

We have developed an automated microscopy-based approach to study cell cycle and DNA-damage signalling with high spatial and temporal resolution. By growing single cells on micropatterns we can reduce the cellular variation depending on differences in cell-to-cell and cell-to-matrix contacts. Moreover, since cells grow on predefined positions we can largely reduce the effect of spatial variation in illumination intensity. We currently use this approach to gather large data sets of immunofluorescence quantifications correlated to live-cell quantifications of biosensors. These datasets will form the basis for experimentally derived mathematical models of mitotic entry and checkpoint recovery.

MODELING REPLICATION KINETICS IN BUDDING YEAST REVEALS THAT REPLICATION TIMING IS REGULATED BY MCM LOADING

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Eukaryotic genomes replicate in define patterns with some parts of the genome replicating early in S phase and other parts replicating later. Replication timing correlates with transcription, chromatin modification, sub-nuclear localization and genome evolution, suggesting an intimate association between replication timing and other important aspects of chromosome metabolism. However, the mechanism of replication timing is currently unknown.

Genome-wide replication kinetics have been mapped in several organisms. The rich datasets that result contain more information than has been extracted by current methods of analysis. We have developed an analytical model that exploits previously unutilized information on the temporal aspects of replication. The model incorporates probabilistic initiation of origins, variable fork-progression rates, and passive replication. We used the model to compute ensemble averages of replication extent and performed least-squares fits to a set of recently published time-course microarray data on *Saccharomyces cerevisiae*. We extracted the distribution of firing times for each origin and found that the later an origin fires on average, the greater the variation in firing times. This trend leads naturally to a model where earlier-firing origins have more initiator complexes loaded and a more-accessible chromatin environment. The model demonstrates how initiation can be stochastic and yet occur at defined times during S phase, without an explicit timing program. Furthermore, we propose that the initiators in this model correspond to loaded MCM complexes and that the timing of origin firing is regulated in by the number of MCM complexes loaded at an origin. Thus, for the first time, our model suggests a detailed, testable, biochemically plausible mechanism for the regulation of replication timing in eukaryotes. Preliminary experimental validation of our model confirms that early firing origins have more MCM loaded. We are currently extending this validation by ChIP-seq genome-wide quantitation of MCM occupancy.

ANALYSIS OF THE PHOSPHOCREATINE SHUTTLE BY MULTISCALE "SLOPPY" MODELLING

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Changes of workload in heart muscle require quick adaptation of oxidative phosphorylation (OxPhos) and energy transport across the cytosol to provide sufficient energy for contractile function. Here we predict the confidence regions of the relative contributions of ATP and phosphocreatine (PCr) to the phosphotransfer system and the damping capability of the system with regard to the large swings in ATP hydrolysis during the cardiac contraction cycle. We combine a "sloppy modelling" ensemble simulation approach (Gutenkunst et al., PloS Comp. Biol, 2007) with multiscale data measured at the level of enzymes and on dynamic response times of OxPhos at the whole organ level. The "PCr shuttle hypothesis" states that the creatine kinase (CK) system is obligatory to transport high-energy phosphate groups from ATP producing sites in mitochondria, by PCr production via the mitochondrial isoform of CK, to produce ATP via the cytosolic isoform of CK in the myofibrils. Basis of this shuttle is the reversible transfer of phosphate by the CK reaction (ATP+Creatine ? ADP+PCr), with transport of PCr between the enzyme isoforms. We analysed the PCr shuttle with a 'skeleton' model containing the major components of the compartmentalized CK system including mitochondrial ATP synthesis and hydrolysis, direct ATP transfer and both CK isoforms (Van Beek, Am. J. Physiol., 2007). The model relies on 24 molecular parameters describing the kinetic behaviour of the system. Most of them had been measured on isolated enzymes or mitochondria, only the mitochondrial outer membrane permeability (PSmom) for ADP and ATP, a key parameter determining the contribution of PCr and ATP to energy transport, was estimated by fitting the response time of OxPhos. From this we predict that PCr transports about 1/3 of all high-energy phosphate groups, which contradicts the "PCr shuttle hypothesis." To investigate the effect of measurement error on this prediction we consider ensembles of possible parameter combinations yielding a range of possible model predictions. In the 'sloppy' modeling approach, the likelihood of a parameter combination being included in the ensemble is proportional to the parameter combination's likelihood to predict the given experimental input data set. The quality of the fit is assessed by a chi-square cost function. The ensemble is sampled from parameter space with Markov Chain Monte Carlo. We took an expanded data set containing the response times of OxPhos to a series of challenges with electrical cardiac pacing rates, combined with data measured on isolated enzymes and mitochondria. From the ensemble we predict that the PCr contribution to the transport of high-energy phosphate groups from mitochondria to myofibrils is with 95% probability between 7 and 40%. Further, we predict damping of oscillations in mitochondrial activity by the CK system: CK inhibition by 98% leads to an increase in amplitude of the ATP synthesis pulsation from 230±80 to 650±150 $\mu M * l^{-1} * s^{-1}$ showing damping by CK activity of the ATP hydrolysis amplitude during systolic cardiac contraction of 3750 $\mu M * l^{-1} * s^{-1}$. Our findings strengthen the hypothesis that CK acts mainly as a high-capacity temporal energy buffer damping energetic pulsations rather than being an essential energy transport system.

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