predictive models of complex systems

POSTER ABSTRACTS

Sunday June 4, 2006

Systems Biology Applied to Peroxisome Biogenesis and Function
Aitchison, John, Institute for Systems Biology

Systems biology approaches are being employed to understand the cellular events involved in the formation, maturation and turnover of functional yeast peroxisomes. Two examples are highlighted:

To identify proteins associated with peroxisomes we have combined classical subcellular fractionation with large-scale quantitative mass spectrometry to identify proteins that specifically enrich with peroxisomes of *Saccharomyces cerevisiae*. Isotope-coded affinity tags and tandem mass spectrometry were used to quantify the relative enrichment of proteins during the purification of peroxisomes. Mathematical modeling of the data from the 306 quantified proteins led to a prioritized list of 70 candidates whose enrichment scores indicated a high likelihood of them being peroxisomal. Several bona fide peroxisomal proteins are shared with other subcellular structures and their relative localization changes with cell state. For e.g. while Rho1p is tethered to membranes of the secretory pathway, it was also recruited to peroxisomes upon their induction. In the peroxisomal membrane, as in other membranes, it appears to mediate membrane fusion, an event associated with peroxisome dynamics and biogenesis.

To decipher the transcriptional regulatory network controlling peroxisome induction we are taking the following approach: 1. Generate transcriptome-profiling data during exposure to fatty acid. 2. Mine the data to identify candidate peroxsiome-related transcriptional regulators. 3. Identify targets of each candidate using two complimentary approaches: a) Large-scale chromatin localization analysis of each factor under conditions of peroxisome induction and repression. b) Compare the transcriptome profiles of deletion strains to that of the wild-type strain for each transcription factor under peroxisome inducing conditions using microarrays. 4. Integrate the data with relevant data in the literature and generate models using network visualization and analysis software termed Cytoscape. 5. Test predictions and refine the models. This approach has revealed the dynamics and modularity of the regulatory response controlling peroxisome induction.

A 3D Mechanical/Biochemical Model of Prokaryotic Plasmid Segregation
Alberts, Jonathan, Center for Cell Dynamics, University of Washington

Experimental observations continue to refine our understanding of the ParM mediated plasmid segregation machinery common to many bacteria with low-count drug-resistance plasmids. Here we present the details of a 3-dimensional object-oriented computational model, simulating both the biochemistry and force generation in this prokaryotic “mitotic spindle”, designed to test hypothesized workings of this system. ParM is a prokaryotic actin homolog with unique filament dynamics: filaments form as two-stranded helical filaments with equal kinetics at each end and occasional catastrophic depolymerization. ParM filaments in this computational model nucleate, polymerize monomer by monomer, and undergo catastrophe at experimentally determined rates. Individual monomer hydrolysis states are simulated, since these states are thought to determine the polymerization/depolymerization behaviors of any individual filament; presently uncertain features of ParM filament dynamics, such as a hydrolysis dependent polymerization cap condition, can thus be tested and fit with experimental data. Filaments ends can “capture” plasmids and in so doing reduce depolymerization rates and/or

modify the polymerization cap condition at the interacting end. Forces in this model arise from the thermal energy of the environment, collisions between filaments, plasmids, and the boundaries of the bacterium, and from linkages between filament ends and captured plasmids. Filament polymerization generates force on captured plasmids and, if unchecked, will eventually locate all plasmids to one end or the other of pill shaped bacteria. We plan to use this model to explore the efficiency and fidelity of ParM plasmid segregation, subject to variation in mechanistic and biochemical assumptions.

Interpretable Modeling of Complex Signaling Networks using Fuzzy Logic

Aldridge, Bree[1,2], Mingsheng Zhang[1,3], Douglas Lauffenburger[1,2,3], Peter Sorger[1,2,3]
[1] Center for Cell Decision Processes, Massachusetts Institute of Technology; [2] Biological Engineering Division, Massachusetts Institute of Technology, [3] Department of Biology, Massachusetts Institute of Technology.

Computational models are important tools for quantitatively exploring complex signaling networks. A major challenge is balancing model specificity and interpretability. Detailed, mechanistic differential equation-based models describe the kinetics of all the interactions between network species but are often too complex to grasp. In contrast, statistics-based modeling techniques, such as those based on Bayesian networks, are easier to conceptualize but only identify influences between network species. Here, we describe an alternative modeling method based on fuzzy logic, which enables comprehensive mechanistic modeling and yet is easy to manipulate and interpret. We use fuzzy logic-based modeling in combination with experimental measurements and differential equation based-models to study crosstalk between EGF, TNF, IGF, and estrogen signaling in human cancer cell lines.

(4) *Towards Quantitative Systems Biology Re-emerging a Powerful Biological Idea, Its Development and Applications*

Ao, Ping Ph.D., Mechanical Engineering and Physics, University of Washington

An overview on my NIH project will be given. It consists of four parts:

1) Solving Robustness Puzzle of Lambda Switch----smallest genetic circuit, and Re-Emerging a Powerful Biological Idea: the adaptive landscape. The stability puzzle of lambda genetic switch has been around for at least 20 years, till 2004, or, to some, till now. The puzzle is not in that any isolated set of biological data could not be explained quantitatively--we know this well from today's synthetic biology progresses that it could, it is in that there are so many experimental data sets at many levels of biological structure hence there is very little room for modelers to adjust their parameters. Since 2000 we have been studying this puzzle, and in late 2002 a breakthrough was made: A quantification of the adaptive landscape deeply rooted in biology was discovered. It is a natural measure of stability and robustness. With its help, the key *in vivo* and *in vitro* difference and the extrinsic noise contribution have been identified. A quantitative agreement with biological experiments has been obtained with a nontrivial experimental later independently confirmed by an in vivo experiment. In the light of current (primitive) biological modeling, it might sound too good to be true. You will have a chance to interrogate one of its main defenders, myself, for such quantitative modeling in biology.

For a review of our effort, X. Zhu, L. Yin, L. Hood, D. Galas, P. Ao, Efficiency, Robustness and Stochasticity of Gene Regulatory Networks in Systems Biology: lambda Switch as a Working Example, http://xxx.lanl.gov/ftp/q-bio/papers/0512/0512007.pdf .

2) Quantification of Adaptive Landscape--Scope of the problem and its difficulty beyond biological sciences. Numerous problems on the quantification of the adaptive landscape in higher dimensions have surfaced. We have made a few important progresses. For example, Chulan Kwon, Ping Ao, and David J. Thouless, **Structure of stochastic dynamics near fixed points,** Proc. Natl. Acad. Sci. (USA) **102** (2005) 13029–13033.

3) Re-Formulation of Darwinian Dynamics--clarifying two profound controversies: Fisher's fundamental theorem of natural selection and Wright's adaptive landscape

4) Current Efforts and Directions--Kinetic modeling of metabolic pathways of AM1 and Cancer as an evolutionary process

Investigating Mechanisms of Activation of Cardiac Energetics during Exercise with in-silico Studies.
L. Zhou, W.C. Stanley, N. Sharma, I.C. Okere, G.M. Saidel, and **M.E. Cabrera**. Center for Modeling Integrated Metabolic Systems, Case Western Reserve University

The heart has the capacity to increase its metabolic rate by 3-5 fold while maintaining the concentrations of ATP, ADP, and inorganic phosphate (Pi) fairly constant. To date, the interaction among mechanisms maintaining ATP homeostasis in myocytes, in spite of changes in workload, has not been elucidated. Experimental evidence suggests that NADH and NAD^+ are also maintained nearly constant with increased cardiac workload. However, current tissue measurements of these regulatory metabolites (ATP, ADP, Pi, NADH, NAD^+) lump cytosol and mitochondria and do not provide dynamic information on their concentration and flux rate changes during the early phase of the low-to-high work transition, thus limiting their inferential value. Our previously published computational model of cardiac metabolism that distinguishes cytosol and mitochondria was modified by incorporating mechanisms of pathway activation (e.g., ATP hydrolysis, glycolysis, dehydrogenases, electron transport chain, oxidative phosphorylation) to simulate the metabolic responses to a step increase in cardiac workload in these subcellular compartments. Model simulations showed that myocardial O_2 consumption, pyruvate oxidation, fatty acids oxidation and ATP generation were all increased with increased workload, while ATP and ADP remained constant. Both cytosolic and mitochondrial NADH/ NAD^+ increased during the first few minutes (40% & 20%, respectively) and returned to resting values by 10-15 min. Furthermore, simulations showed that an altered substrate selection -induced by either exercise or diabetes- affected cytosolic NADH/ NAD^+, but had minimal effects on mitochondrial NADH/ NAD^+, myocardial oxygen consumption, or ATP production. In conclusion, these results support the concept of parallel activation of metabolic processes generating reducing equivalents during a step increase in cardiac work load and suggest the existence of a transient increase in the mitochondrial NADH/ NAD^+, which is independent of substrate supply.
Key words: exercise, heart, lactate, mitochondria, modeling.

In-Silico Studies of Cellular Metabolic Dynamics in Skeletal Muscle
Ranjan K. Dash [1,3], Yanjun Li [1,2], **Gerald M. Saidel[1,2]**, **Marco E. Cabrera**[1,2,3]
[1]Center for Modeling Integrated Metabolic Systems; [2]Departments of Biomedical Engineering and Pediatrics; [3]Case Western Reserve University

The control mechanisms of cellular metabolism and energetics in skeletal muscle that become evident in response to physiological stresses such as reduction in blood flow and oxygen supply to mitochondria can be quantitatively analyzed using a computational model. The analysis of dynamic responses from a model of metabolism and energetics in the perfused tissue/cells can provide insights into the mechanisms of metabolic regulation that complement experimental studies. For this purpose, a novel large-scale computational model of skeletal muscle cellular metabolism and energetics is developed to describe the dynamic responses of key chemical species and reaction fluxes to muscle ischemia. The model, which incorporates key transport and metabolic processes, is based on dynamic mass balances of the chemical species in both capillary blood and tissue cells domains. The metabolic reaction fluxes in the cellular domain are expressed in terms of a general "phenomenological" Michaelis-Menten equation involving the ATP/ADP and NADH/NAD^+ energy controller ratios. The large number of unknown model parameters is evaluated by minimizing the differences between the model outputs and available experimental data using a nonlinear constrained-based reduced gradient optimization algorithm. With these parameter values, the model is able to simulate dynamic responses to reduced blood flow and oxygen supply to mitochondria associated with muscle ischemia of several key metabolites and metabolic fluxes that can be measured and others that cannot be measured with current experimental techniques.

Mechanical Coupling and Force Transmission in Growth Cone Filopodia
Chan, C., and D. Odde, Department of Biomedical Engineering, University of Minnesota

In the developing nervous system, the growth cone motile machinery plays a critical role in wiring very specific connections at relatively long distances. Essential to this machinery are filopodia, long, thin, actin-based protrusions thought to play an exploratory and guidance role in this behavior. While much work has focused on the components and regulatory mechanisms of this machinery, quantitative descriptions of how these components might work together to engage substrates and transmit forces continue to be lacking. To address these problems, a stochastic computer simulation based on motor-clutch motility was created. These simulations reveal for the first time, detailed, quantitative descriptions of how this machinery may operate and how its operation might be affected by clutch properties, the mechanics of the microenvironment, as well as externally applied forces. Furthermore, recent experimental work on the filopodia of embryonic chick forebrain neurons mirror predictions made by simulations supporting the validity of motor-clutch action in this system. Future work will focus on fully mapping the behavior of this system in parameter space as well as developing experimental systems to test these predictions in neuronal growth cones.

Mps1 phosphorylation of the Dam1 kinetochore complex target kinetocore plus-ends
Davis, Trisha[1], Michelle Shimogawa[1], Beth Graczyk[1], Melissa K. Gardner[3], Susan E. Francis[1], Michael Ess[1], Jeffrey N. Molk[4], Cristian Ruise[5], Sherry Niessen[5], John R. Yates, III[5], Eric Muller[1], Kerry Bloom[4], David J. Odde[3], Trisha Davis [1, 2], [1]Dept of Biochemistry, University of Washington and [2]Program in Molecular and Cellular Biology, University of Washington, [3]Department of Biomedical Engineering, University of Minnesota, [4]Dept of Biology, University of North Carolina, Chapel Hill, and [5]Dept of Cell Biology, Scripps Research Institute.

Duplicated chromosomes are segregated to daughter cells by the mitotic spindle during cell division. The assembly of the bipolar spindle with each sister chromatid properly bioriented occurs in multiple steps. First, chromosomes are captured by lateral attachments to the spindle microtubules via the kinetochores. By metaphase, the kinetochores are moved to the plus-end of microtubules and bioriented. By combining yeast genetics, cell biology and computational analyses of mitotic spindles, we found that Mps1 phosphorylation of the Dam1 kinetochore component is required for kinetochore attachment to the plus-ends of microtubules. Surprisingly, plus-end attachment is not required for i) chromosome biorientation ii) maintenance of tension between sister kinetochores or iii) chromosome segregation.

Phosphoinositides and Rho proteins Spatially Regulate Actin Polymerization in Motile Cells
Dawes, Adriana and Leah Edelstein-Keshet, Dept. of Mathematics, University of British Columbia

Polymerization of the protein actin generates sufficient force to propel a cell in response to an external signal. In this poster I will present a model of actin polymerization dynamics that incorporates the activity of phosphoinositides (membrane-bound lipids that mediate sensing of external signals, PIs) and Rho proteins (regulators of actin dynamics). The model is based on experimental evidence indicating how PIs and Rho proteins interact with each other and how they affect actin dynamics. The model generates behaviours consistent with experimental observations of both normal and mutant cells and suggests possible mechanisms for recent observations. I propose testable hypotheses than can be used to further refine the model and shed light on the regulatory processes involved in cell motility.

How might microtubules control cortical contractility during cytokinesis?
Foe, Victoria, George von Dassow, and Garrett Odell, Center for Cell Dynamics, University of Washington.

Our micromanipulation experiments, moving the mitotic apparatus inside live anaphase/telophase echinoderm blastomeres, shows that the equatorial zone of RhoA activation (detected by GFP-rhotekin) is being continuously re-generated, with a time scale of tens of seconds, even as cytokinesis proceeds (also see Bement & von Dassow, 2005). We find a tight correlation between cortical activation of RhoA and cortical localization of myosin II that is phosphorylated at RLC serine19. In three echinoderm species we find that only a small fraction of total myosin II is ser19 phosphorylated

and this fraction is cortically localized. When we block RhoA activation with C3 no cortical accumulation of Pser19 myosin (or of any myosin II) occurs. During normal cell cycle progression, cortical Pser19 myosin levels remain uniform, low, and relatively constant from interphase through metaphase, *decrease* over the entire cortex during anaphase prior to furrow initiation, then increase locally to high levels at the cell equator. We emphasize that the preamble to cytokinesis entails a global loss of cortical Pser19 myosin, which begins at the onset of anaphase and reaches its nadir prior to Pser19 recruitment to the cytokinetic furrow.

Recent studies suggest a positive role for stable microtubules in furrow induction (Shannon et al., 2005; Canman et al., 2003). By treating echinoid blastomeres for five minutes with 20 uM nocodazole (well above the concentration needed to depolymerize most of this cell's microtubules) we have revealed a previously undescribed transient population of stable microtubules that forms during anaphase, converts into an array that includes the central spindle plus some astral microtubules (the majority of which stable microtubules form on the side of the centrosome that faces the chromosomes), and disappears as abscission completes. 3-D reconstructions of nocodazole-treated purple urchins blastomeres show a coincidence between where stable astral microtubules contact the cortex and where localized myosin phosphorylation first occurs. Treatment of purple urchin blastomeres with 20uM nocodazole immediately after cells traverse the spindle checkpoint either thwarts both the whole-cell myosin dephosphorylation during anaphase and subsequent equatorial myosin recruitment, or else it increases the width of the myosin-enriched equatorial myosin band and the concentration of Pser19 myosin in this band. The above observations suggest that stable microtubules, but not dynamic microtubules, are conveying a signal that causes the high-level RhoA activation at the cell equator and imply that the whole-cortex anaphase myosin *dephosphorylation* and the localized high-level myosin phosphorylation at the presumptive furrow are somehow interconnected.

We suspect it is kinesin-mediated transport of RhoGEFs along stable astral microtubules (where a high GEF concentration would lead to a high concentration of activated RhoA, which in turn activates a ring of myosin II) that explains how the mitotic apparatus determines the future location of the contractile ring. We have begun using an individual-based computer simulation to explore the difference stable vs. dynamic microtubules could have for establishing a sharply delimited band of RhoA activation. This hinges on whether the motor transport and RhoA/myosin-phosphorylation biochemistry dominate or are undone by dissipative effects. This is both a qualitative issue (hinging on the biochemical/physical properties of kinesins, microtubules, RhoA activation, myosin phosphorylation etc) and a quantitative issue, resolvable we believe, only by using individual-based computer simulations of the sort our center is developing. If kinesins are responsible for conveying the GEFs that activate RhoA, our modeling shows that diffusion will undo any signal concentration that normally behaved kinesins set up too fast to establish a sharply bounded active RhoA zone. However, our preliminary computer exploration of this issue shows that stable microtubules combined with kinesins that hang onto microtubule tips is one way to build a signaling mechanism that can dominate diffusive attenuation to maintain a RhoA zone with the requisite sharpness and stability. Simulations of our assumptions lead to a reduction in diffusible RhoGEFs as microtubules grow out (predicting the observed reduction in active cortical RhoA & myosin during normal anaphase progression), and reproduce the over-activation of the equatorial myosin zone we observe following nocodazole-treatment during early anaphase.

Control of Signaling in a MAP-by an RNA-binding Protein
Galitski, Timothy, Susanne Prinz, Christine Aldridge, Stephen Ramsey, James Taylor, Bruz Marzolf, Institute for Systems Biology

The yeast MPT5 gene encodes a PUF-family RNA-binding protein. We found that it is a repressor of yeast cell differentiation to the filamentous growth form. MPT5 represses the protein levels of two filamentation MAP-kinase pathway components, the Ste7 MAP kinase and the Tec1 transcriptional activator, and inhibits signaling through the pathway. He results suggest that MPT5 prevents inappropriate filamentation by restricting the signaling capacity of the pathway.

Modeling and Analysis of a Sub-Resolution Spatial Gradient in Spindle Microtubule Dynamics
Gardner, Melissa[1], David Odde[2], Chad G. Pearson[2]; Leocadia V. Paliulis[3]; Kerry Bloom[3]; E. Salmon[3]; and David J. Odde (1) [1]University of Minnesota, [2]University of Colorado at Boulder, [3]University of North Carolina at Chapel Hill

Through an integrated approach utilizing both digital fluorescence microtubule dynamics that can explain the congression of kinetochores to a metaphase configuration during budding years mitosis. This model predicts that there would be a spatial gradient in microtubule turnover in the yeast metaphase spindle, such that regulation of kinetochore microtubule dynamics promotes plus-end turnover specifically in the locations of metaphase kinetochore clustering. In order to test this prediction, we developed a high-resolution fluorescence recovery after photobleaching (FRAP) method that allowed for spatial sampling in ~65 nm intervals along the length of the mitotic spindle. Using this method, we observed experimentally a gradient in plus-end microtubule dynamics are highest near kinetochores and lowest near the spindle poles. A beta-tubulin mutant that has decreased plus-end dynamics preserves the spatial gradient in tubulin turnover on a slower time scale, indicating that kinetochore-based regulation of microtubule dynamics can mediate pre-anaphase chromosome congression even in the presence of altered intrinsic microtubule dynamics.

Mechanisms of Chromosome Alignment and Spindle Fusion: Insights from Spindle Micromanipulation
Gatlin, Jesse C. [1], Lisa A. Cameron[1], Aaron C. Groen[2], Timothy J. Mitchison[2], and E. Salmon[1]
[1]Department of Biology, University of North Carolina, Chapel Hill, [2]Department of Systems Biology, Harvard Medical School

Meiosis II bipolar spindles with replicated sister chromosomes were assembled in *Xenopus* egg extracts. A previous study demonstrated that two spindles brought together with microneedles will fuse into one bipolar spindle (Mitchison *et al.*, 2000). We began to explore the microtubule (MT) and motor mechanisms that align and fuse two spindles together by positioning spindles with their interpolar axes parallel to each other. We recorded spindle assembly by polarization optics, chromosomes by DAPI fluorescence, and spindles poles labeled with Alexa-488 conjugated anti-NuMA antibody. If proximal ends of the spindles were overlapped by at least 1 μm of spindle length, then the two spindles slid parallel to their axes until the chromosomes and poles were aligned. Sliding occurred at constant velocity (1.2 ± 0.4 μm/min). Spindle alignment also occurred by a second mechanism. If spindles were positioned with no overlap between poles, then the adjacent poles came together and spindles pivoted around their shared pole, "jackknifing" into a bipolar spindle. In other experiments, spindles were positioned side-by-side about 10-20μm apart with various orientations of their interpolar axes. They were pulled together by MT arrays that formed between polar regions and then aligned by sliding. To test for the origin of the pulling forces, we created monopolar spindles by biochemical inhibition of the Eg5 kinesin. When two monopoles were placed within about 30 μm from each other, a central spindle of overlapping MTs formed between the poles which then shortened and disappeared as the poles came together. Velocity of the converging poles was initially fast (1. 7 ± 0.7 μm/min) and slowed as the two poles came closer together. Central spindle formation and pulling were blocked by 200μM orthovanadate, indicating that both are caused by cytoplasmic dynein. These data suggest that "pulling in" forces produced by cytoplasmic dynein play a major role in the alignment and fusion of two spindles into one. Supported by GM24364 and GM60678.

Regulation of Rho GTPase Activty
Jilkine, Alexandra, A. Maree, and L. Edelstein-Keshet, Dept. of Mathematics, University of British Columbia

Cdc42, Rac, and Rho are small GTPases known to play a central role in signal transduction to the actin cytoskeleton. These proteins regulate cell motility, by affecting actin nucleation, uncapping, depolymerization, and acto-myosin contractility. Studies of crosstalk, and mutual feedbacks, in these three proteins, have led to a number of proposals for their interaction. At the same time, observations of the spatio-temporal dynamics of Rho-family proteins give evidence of spatial polarization and mutual exclusion between Cdc42/Rac and Rho. We formulate a mathematical model to account for such observations, based on the known underlying biology of

these proteins. We first investigate which of the crosstalk schemes proposed in the literature is consistent with observed dynamics, and then derive a simple module that can correctly describe these dynamics (assuming crosstalk is mediated via Rho GEF's). We show that cooperativity is an essential ingredient in the interactions of the proteins, and that fast spatial segregation is related to bistability of the underlying ODE's. The fast diffusion of the inactive forms of these proteins is essential for stabilizing the transition fronts in the PDE formulation of the model, leading to robust spatial polarization, rather than traveling waves. Our module has been used elsewhere in a 2D model for cell polarization and motility.

Regulation and Modeling of the Drosophila Endocycle Network

Kim, Kerry[1], Vuong Tran[2], Ryan Gile[1], Garret Odell[1], George von Dassow[1], and Bruce Edgar[2], [1] Center for Cell Dynamics, University of Washington, [2] Fred Hutchinson Cancer Research Center

The endocycle is an abbreviated cell cycle in which successive rounds of S phase occur without mitosis; thus DNA content doubles with each cycle. Temporal oscillations of Cyclin E (CYCE) protein trigger entry into S phase in endocycling Drosophila cells. These CYCE oscillations are controlled and regulated by a network which includes E2F, DP, and retinoblastoma (RBF). We wish to determine how the interactions between these genes produce CYCE oscillations. We quantified CYCE and E2F levels in endocycling cells and found E2F levels peaked before S phase then disappeared as CYCE levels rose. E2F and CYCE oscillations continued with little change when S-phase entry was blocked with hydroxyurea. This raises the question of whether CYCE oscillations could arise due to known regulation by E2F and RBF. As a rigorous test of this, we reconstituted the known interactions between E2F, RBF and CYCE in a computational model to see whether it could reproduce the qualitative and quantitative features measured in experiment. A random search of parameter space revealed that the model can reproduce several of the qualitative and quantitative features we observe in endocycling cells.
[1]Supported by NIGMS: 5 P50 GM66050 and NSF: MCB0090835.

Exploring Topology Space to Identify a Signaling Network that Recapitulates Mesectoderm Specification in Drosophila

Konieczka, Jay, H., Leo B. Lopes, and Parker B. Antin, University of Arizona

Notch signaling plays a role in establishing the expression domain of single-minded (sim), a transcription factor that defines two single-cell rows of mesectoderm along the anterior-posterior axis of the Drosophila embryo. sim expression is established by non-cell-autonomous activation of sim by snail, a transcriptional repressor, via Delta-Notch signaling. Interestingly, sim expression is also repressed by snail in a cell-autonomous manner. To model this system, we adapt a computational model that describes the role of Notch signaling in lateral inhibition during neurogenesis (Meir et al., 2002). We expand a subnetwork of the Notch neurogenesis model to include the transcription factors sim and snail as well as other suspected players. Since correctness of the new model is unknown, we explore network topologies using a heuristic based on two observations: first, since establishing this expression domain is critical for development, we reason that robustness to parameter settings is a good measure of network correctness; second, previous work has shown that network neighbors in topology space share similar robustness metrics. Building on these ideas, our heuristic uses statistical tests to quickly evaluate many solutions in a neighborhood.

Monday June 5, 2006

Numerical Estimation of Progesterone Transcription in the EGFR Pathway
Marquez-Lago, **Tatiana, T.,** Stanly L. Steinberg, Alex Slepoy, Kimberly Leslie, University of New Mexico, Sandia National Laboratories

Optimization of progesterone transcriptional activity has recently been thought to be an efficient method for controlling endometrial cell growth. As simple as it may sound this goal is not trivial, as the reactions themselves are nonlinear and ubiquitin can break down different forms of progesterone receptors (PrRA and PrRB) throughout the pathway, thus representing a bottleneck for mRNA formation. But, how can one determine the ranges of concentrations of the molecular species involved in obtaining such an optimal transcription? We approached this question with the numerical simulation of the reactions involved in the pathway using Chemcell(c) (Sandia National Laboratories). This software treats molecular species as particles that move via random motion, and allows for user-specified creation of cellular compartments, molecular species, initial concentrations, diffusion, and reaction rates. We consider a parametric analysis of diffusion and reaction rates. We obtained initial approximations from deterministic systems in single and multiple compartments. For well-mixed systems we compare deterministic results with stochastic variational bounds, whereas spatial heterogeneous hypothesis (e.g. hotspots and storage areas inside the nucleus) are simulated stochastically only. Numerical results for simulations of the EGFR-pathway will be presented, as well as partial validation of simulations (comparison with experimental biological data).

Robustness and Evolution of Vulva Development in C.elegans: an In Silico and Experimental Approach
Milloz, Josselin[1], I. Nuez[1], E. Meir[2] and Marie-Anne Felix [1]
[1]Jacques Monod Institute – CNRS; [2]Symbiotic Software for Teaching and Research

Many organisms produce phenotypes that are robust to stochastic noise and to a range of environmental variations. Such stability seems to arise from properties of the underlying genetic networks that act during development. Importantly, this environmental robustness may also result in robustness to some genetic variations, thus allowing the evolution of developmental processes without any phenotypic change (silent or cryptic evolution). Here, we are addressing two questions: i. what are the robustness properties of the system; ii. How does the system evolve? We used vulva formation in *C. elegans* as an example of a well-characterized developmental system. In this case, the final cell fate pattern is almost invariant within and among wild isolates. Out of 6 competent cells (p(3-8.p), only three cells normally adopt a vulval cell fate: P6.p 1_ fate), P5p and P7.p (2_ fates). This pattern formation and its robustness relies on three signaling pathways (Ras, Notch and Wnt pathways), which show partial redundancies and various other buffering mechanisms. I.E. Meir and M.-A. Félix have developed an in silico model of the vulva developmental genetic network to assay robustness. The network is described through sets of differential equations using 'Ingénue'[1]. By picking parameter sets randomly and looking at the resulting fate pattern, they proved that the target fate pattern was very easy to reach and thus was robust to parameter variation; iii. In order to unravel potential cryptic genetic differences between wild *C. elegans* strains, we 'debuffered' the system. Practically, mutations that affect the activities of the three pathways were introduced by repeated backcrosses into six wild *C. elegans* isogenic backgrounds. The effect of a given mutation varies significantly between different wild genetic backgrounds. These unraveled differences suggest that the vulva patterning network has evolved within the *C. elegans* species in the absence of any change in the final phenotype. As a next step, introducing noise into the model should allow us to both assay robustness against it and mimic partially penetrant phenotypes. We may further be able to map the different *C. elegans* wild strains into different parts of parameter space of solutions and try to draw the more likely 'evolutionary pathways' between these wild populations. [1]: Meir E., Munro E.M., Odell G.M., von Dassow, G. Ingenue: a versatile tool for reconstituting genetic networks, with examples from the segment polarity network. J Exp Zool, (2002) 15;294(3):216-51.

Control of TGF-beta Signaling
Oscamou, M., D. Clarke, X. Liu, and M. Betterton, University of Colorado

The TGF-beta family of proteins regulates a number of cellular processes including cell proliferation, differentiation, apoptosis, and migration. The general steps by which the TGF-beta induced signal is transmitted from cell receptors to target genes have only recently been determined and key control points of the signaling network are still under debate. We use mathematical modeling to predict the control points of the network. A key difficulty in modeling the TGF-beta signaling system is that most of the rate constants for the reactions in the network are unknown, or vary from cell to cell. Following the recent work of D. Clarke et al., K.S. Brown *et al*., and D. Battogtokh, we use a Monte Carlo method to identify an ensemble of possible rate constants that result in dynamic behavior consistent with published experimental data. Analysis of the ensemble identifies key control points in the TGF-beta signaling network. We compare the results to the parameter sampling results for this model found by Clarke et al.

Quantitative Measurement and Modeling of the DNA Damage Response
Ostheimer, Gerard, J. , Alexandra Gardino, Ericka Noonan, Dharini Shah, Andrea Tentner, Michael Yaffe, Leona Samson and Douglas A. Lauffenburger, Center for Cell Decision Processes, Massachusetts Institute of Technology

Mammalian cells have an extensive DNA damage signaling machinery that detects and coordinates the response to DNA damage. Cells either repair damage, activate cell cycle checkpoints, which in turn permit repair, or apoptosis, as a function of the nature and extent of the damage. We are curious about how cells "decide" their fate after their DNA has been damaged. The molecular machinery responsible for this cellular decision process is a network of protein kinases and phosphatases the biology and biochemistry of which are being increasingly well characterized. Nonetheless, exactly how the information from various stress response pathways are merged such that the cell commits to a given outcome is unclear. Our goal is to develop predictive, computational models of the DNA damage signal transduction network that will permit insight into this cell decision process. These models will be based on extensive experimental observation of the activity of the DNA damage signaling network as a function of time after genotoxic damage. The activity of kinases involved in regulating the cellular response to DNA damage will be measured using high-throughput kinase assays and quantitative immunoblotting. In our immunoblotting studies we will monitor both the phosphorylation of the kinase of interest and phosphorylation of its targets. For example, the activity of ATM will be monitored at dozens of time points after damage by measuring the phosphorylation state of ATM targets such as p53 and Chk2 as well as by measuring the phosphorylation state of ATM itself. An exciting approach currently being developed by us is the use of multi-colored flow assisted cell sorting to simultaneously monitor the phosphorylation states of multiple proteins in individual cells. Concomitant with kinase measurements, kinase localization, cell cycle progression and apoptosis will likewise be measured. These data sets will be merged to generate predictive mathematical models of the cellular decision process. We will then validate and refine these models through selective, quantitative perturbations of the signaling network and measurements of perturbed network function. These quantitative perturbations will be incurred either pharmacologically or by knocking down gene expression levels using RNA interference.

Altering ActA Polarity Constrains the Nature of the Listeria-Actin Tail Tether: Concurrent Experiment and Simulation
Rafelski, Susanne M., Jonathan B. Alberts, Garrett M. Odell, Center for Cell Dynamics, University of Washington

The surface protein ActA is the only bacterial protein required for the initiation and maintenance of the actin-based motility of the pathogen *Listeria monocytogenes* within its host cell. The ActA protein has a polar distribution on the surface that varies between individual bacteria, falling into several distinct natural classes and affecting both bacterial speed and motility initiation. To understand how the degree of ActA polarity affects motility we have pursued a combined approach, using both *Listeria in vitro* motility experiments and a detailed

biochemical/mechanical simulation. We can experimentally manipulate ActA distributions by altering bacterial growth and ActA expression rates, creating a mixture of bacteria that exhibit a range of more or less polarized ActA distributions. We visualize and measure ActA on individual bacteria using an ActA-RFP fusion and then quantitatively parameterize the polarity of ActA surface distributions. Multivariate analysis reveals a strong dependence of bacterial speed on both the total amount of ActA and ActA polarity; there is a positive correlation between speed and polarity. Further, bacteria with too little or too much ActA display slower speeds, suggesting an optimal range of ActA levels for rapid motility. Computationally, we can map the measured ActA distributions onto simulated bacteria and gather data on the resulting simulated motility. Adjustment of the mechanical properties of the bacterium-actin tail tether allows the simulation to imitate our experimental results. The sensitivity of bacterial speed to ActA polarity modulated by the tether characteristics suggests that ActA along the sides of bacteria performs competing functions; promoting autocatalytic actin growth and rapid movement while simultaneously providing more ActA molecules and actin filaments for increased attachment between bacterium and tail, slowing movement. The relative strength of these two functions then determines the overall effect of ActA polarity on bacterial speed. To test this hypothesis, we are investigating the mechanical and chemical properties of the ActA-actin tail tether that best allow the simulation to replicate our experimental results. We are concurrently performing experiments using ActA mutants with altered VASP interaction to determine the degree to which VASP, a molecular candidate for the tether, modulates the relation between ActA polarity and bacterial motility.

Cortical Actomyosin Dynamics and the Maintenance of PAR Polarities in the C.elegans Embryo
Schoff, Chris, Howard Clarke, and Ed Munro, Center for Cell Dynamics, University of Washington

The *C.elegans* zygote polarizes in response to a transient cue associated with the sperm centrosomes/microtubule organizing center (MTOC). Anterior-Posterior polarity is established during interphase when the sperm MTOC triggers an asymmetrical actomyosin contraction and cortical flows that carry the conserved polarity determinants PAR-3/PAR-6/aPKC to the anterior and allow others (PAR-1 and PAR-2) to associate with a complementary posterior domain. However, the mechanisms that maintain PAR asymmetries between the end of interphase (when the cue disappears) and the onset of cytokinesis at anaphase remain unclear. Here we explore how actomyosin assembly and activity are involved in the maintenance of PAR polarities.

During the maintenance phase, both F-actin assembly and myosin recruitment are biased to an anterior domain defined by high levels of PAR-3/PAR-6/PKC-3, and these are closely associated with persistent anterior-directed cortical flows. Myosin recruitment and cortical flow during maintenance absolutely require the small GTPase CDC-42 and it's downstream effector, MRCK (Myotonic dystrophy kinase-related Cdc42-binding kinase), a known activator of myosin contractility. Asymmetrical recruitment requires posteriorly localized PAR-2. CDC-42/MRCK-dependent cortical flows are not required for the maintenance of normally established PAR asymmetries, but they can restore asymmetries in mutants that fail to establish them during interphase.

Anterior-biased F-actin assembly during maintenance requires components of the conserved ARP-2/3 complex, which promotes the assembly of branched F-actin meshworks. ARP-2/3 is not required for the establishment of PAR polarities, but depletion of ARP-2/3 components leads to hyper-contraction and mechanical destabilization of the anterior PAR-3/PAR-6/PKC-3 cap during the maintenance phase, and a corresponding anterior extension of the posterior PAR-2 domain. Interestingly, mutations in the posterior PAR protein PAR-1 also lead to hyper-contraction of the anterior cap and simultaneous removal of PAR-1 and ARP-2 produce a strongly synergistic effect. We hypothesize that robust maintenance of PAR asymmetries requires two redundantly acting mechanisms: One involves conserved cross-inhibition among anterior and posterior PAR proteins; the other involves the asymmetrical contraction of actomyosin against an anterior domain that is stiffened by the assembly of a dense network of branched actin filaments.

Prediction of Loading Induced Bone Formation
Srinivasan, Sundar, Brandon J. Ausk, Ted S. Gross, Orthopaedic Science Laboratory, Orthopaedics and Sports Medicine, University of Washington

Cell signaling induced during a single bout of loading (order of 100 s) guides a sweeping cascade of complex cellular events that influence bone tissue formation for days and weeks. To begin to explore this process, we developed a parametric agent based model (ABM) for real-time Ca^{2+} signaling induced within the bone cellular network during a single brief bout of loading. Given that intracellular Ca^{2+} fluctuations are high fidelity events that control numerous downstream cellular events, we hypothesized that real-time Ca^{2+} signaling predicts downstream tissue level bone formation induced by mechanical loading. To test this hypothesis, we explored the parametric ABM via simulated annealing. Within 25,000 iterations, we found that model simulations of real-time Ca^{2+} signaling (order of seconds) could be related to bone formation induced 3-wks later by 10 different loading protocols in the murine skeleton (rms error ~ 11%, max error < 20%). To validate the model, we predicted bone formation induced by 2 additional 'extrapolative' protocols not included in parameter estimation and determined bone formation induced by this protocol via in vivo animal experiments. In support of our hypothesis, we found that the ABM for real-time Ca^{2+} signaling could accurately predict bone formation induced by the two new protocols (rms error < 10%). Significantly, this ABM provides the first computational tool for quantitative prediction and design of mechanical loading protocols and represents an initial framework for the exploration of how mechanotransduction functions within bone.

Sources of Apoptotic Heterogeneity in Clonal Cell Populations
Spencer, **Sabrina L.**, Peter K Sorger, Center for Cell Decision Processes, Massachusetts Institute of Technology

In death receptor-mediated apoptosis, a network of regulatory proteins controls the transmission of the pro-death signal from receptors on the cell surface to effector caspases. These effector caspases then bring about self-destruction of the cell. In a homogenous clonal cell population, cells display great heterogeneity in the time interval between death ligand stimulation and apoptosis, referred to as t-delay. This project explores the sources of heterogeneity in t-delay. Analysis of live-cell microscopy experiments suggests that t-delay is not purely stochastic, but rather is partly deterministic. In these experiments, two daughter cells that shared the same parent in the previous round of cell division die more closely in time to one another than two random cells. In other words, daughter cells have a smaller delta-t delay than random pairs. Thus, knowing the death-time of one cell allows more accurate determination of the death time of the other daughter cell, revealing that there exists some deterministic component to t-delay. The fact that daughters from a recent division have a more similar death time than daughters from an earlier division supports the working hypothesis that this effect is due to similar epigenetics of recently divided cells. We are currently investigating whether cell cycle stage contributes significantly to the observed heterogeneity in t-delay and the extent to which the main apoptotic proteins influence this heterogeneity. An experimentally parameterized differential equation model of the apoptotic pathway guides predictions about sources of heterogeneity and aids in interpreting complex experimental results

Large-Scale Spatial Coordination of Actin Meshwork Flow in Rapidly Moving Cells
Wilson, Cyrus[1], Patricia T Yam[1], Lin Ji[2], Gaudenz Danuser[2], Julie A. Theriot[1]
[1]Stanford University; [2]The Scripps Research Institute

The persistent movement of crawling cells depends on the proper spatial coordination of actin polymerization, actin depolymerization and myosin contraction, with actin polymerization biased toward the cell's leading edge and myosin contraction strongest at the rear. In the case of a motile cell, the entire cell coordinate system moves relative to the frame of reference in which the cell is initially observed. We use a rapid, non-iterative image cross-correlation approach to track cell rotation and translation, which is then used to define the relationship between cell and lab reference frames. This enables us to apply high-resolution tracking techniques including fluorescence speckle microscopy to measurements of the actin meshwork in rapidly moving cells such as fish keratocytes. We characterize, in both lab and cell reference frames, the coordination of F-actin flow dynamics across regions of

cells undergoing different movement behaviors such as transient and sustained turns, and in the presence of drugs such as the myosin-II inhibitor blebbistatin. We find that cichlid keratocytes treated with blebbistatin exhibit an accumulation of actin filaments at the rear and a slight decrease in speed. Blebbistatin also eliminates the inward (perpendicular to cell movement) flow of F-actin in the keratocyte rear and drastically reduces traction forces on deformable gelatin substrates. Notably, cichlid keratocytes moving at steady state do not lose polarity or stop moving when treated with blebbistatin alone. However, when jasplakinolide, which slows actin depolymerization, is combined with blebbistatin, cell motion and actin movement cease. This arrest cannot be achieved in these cells by treatment with jasplakinolide alone, but is a synergistic effect of the two drugs. Thus, continued steady state forward motility requires actin meshwork disassembly in the rear; this disassembly can be driven either by myosin-contraction dependent or contraction-independent processes.