Banff International Research Station
for Mathematical Innovation and Discovery

Mathematical Biology of the Cell: Cytoskeleton and Motility
July 31 – August 5, 2011

MEALS

*Breakfast (Buffet): 7:00 – 9:30 am, Sally Borden Building, Monday – Friday
*Lunch (Buffet): 11:30 am – 1:30 pm, Sally Borden Building, Monday – Friday
*Dinner (Buffet): 5:30 – 7:30 pm, Sally Borden Building, Sunday – Thursday
Coffee Breaks: 2nd floor lounge, Corbett Hall
***Please remember to scan your meal card at the host/hostess station in the dining room for each meal.**

MEETING ROOMS

All lectures will be held in Max Bell 159 (Max Bell Building accessible by walkway on 2nd floor of Corbett Hall). LCD projector, overhead projectors and blackboards are available for presentations. *Note that the meeting space designated for BIRS is the lower level of Max Bell, Rooms 155-159. Please respect that all other space has been contracted to other Banff Centre guests, including any Food and Beverages in those areas.*

SCHEDULE

Sunday

16:00	Check-in begins (Front Desk – Professional Development Centre - 24 hours)
17:30-19:30	Dinner
20:00	Informal gathering in 2nd floor lounge, Corbett Hall Beverages and snacks are available on a cash honor system.

Monday

7:00-8:30	Breakfast
8:30-8:45	**Welcome and Introduction**
8:45-9:25	**Alexander Verkhovsky**, EPFL Lausanne *Interplay between cytoskeletal forces, membrane tension, and hydrostatic pressure in rapidly migrating cells*
9:25-10:05	**Jay Tang**, Brown University *A multiple spring model that predicts bipedal motion of crawling cells*
10:05-10:30	Coffee Break
10:30-11:10	**Alex Mogilner**, University of California, Davis *Mechanical strategies for cell crawling*
11:10-11:50	**Denis Wirtz**, Johns Hopkins University *Cell migration in 3D*
11:50-12:30	**Kinneret Keren**, Technion University *Membrane tension in rapidly moving cells is determined by the balance between actin network assembly, adhesion and contraction*
12:30-13:30	Lunch
	Free Afternoon
17:30-19:30	Dinner
19:30-20:10	**Leslie Loew**, University of Connecticut *Modeling Actin Dynamics: Why the Details Matter*

20:10-20:50 **Sean Sun**, Johns Hopkins University
Cytoskeletal dynamics and mechanosensation of adherent cells
20:50-21:30 **Paul Janmey**, University of Pennsylvania
Driving cytoskeletal remodeling by extracellular matrix mechanics

Tuesday

7:00-8:30 Breakfast
8:30-9:10 **Jennifer Ross**, University of Massachusetts Amherst
Controlling Microtubules Through Severing
9:10-9:50 **Melissa Gardner**, University of Minnesota
Microtubule Length Regulation by Depolymerizing Kinesins
9:50-10:30 **David Odde**, University of Minnesota
Rapid Microtubule Self-assembly Kinetics
10:30-10:55 Coffee Break
10:55-11:35 **Holly Goodson**, University of Notre Dame
Biological insights from computational modeling of microtubule dynamics
11:35-12:15 **David Sept**, University of Michigan
Microtubule mechanics at varying length scales
12:15-13:30 Lunch
13:00-14:00 Guided Tour of The Banff Centre; meet in the 2nd floor lounge, Corbett Hall
14:00 Group Photo – meet on the front steps of Corbett Hall
14:15-16:30 Poster Session
Max Bell Complex
17:30-19:30 Dinner
19:30-20:10 **John Condeelis**, Albert Einstein College of Medicine
Scaling and amplification of signals during tumor cell migration and dissemination in breast tumors
20:10-20:50 **Herbert Levine**, University of California San Diego
Eukaryotic Chemotaxis in Dictyostelium - Getting from the signal to the mechanics
20:50-21:30 **Orion Weiner**, UCSF
Mechanical tension spatially restricts signals to the leading edge during neutrophil migration

Wednesday

7:00-8:30 Breakfast
8:30-9:10 **Jennifer Zallen**, Sloan-Kettering Institute
Shaping the embryo: Cellular dynamics in development
9:10-9:50 **Karen Oegema**, Ludwig Institute for Cancer Research
Using the C. elegans embryo to dissect cell division mechanisms
9:50-10:30 **Adriana Dawes**, University of Alberta / Ohio State University
Negative curvature and cortical thickening may help position the Par protein boundary in the early C. elegans embryo
10:30-10:55 Coffee Break
10:55-11:35 **Inke Näthke**, University of Dundee
Early changes in tissue architecture in colorectal cancer
11:35-12:15 **Arpita Upadhyaya**, University of Maryland
Spreading itself thin: Biophysics of cell junctions in the immune system
12:15-13:30 Lunch

Free Afternoon

17:30-19:30 Dinner

19:30-20:10 **Thomas Pollard**, Yale University
Quantitative Analysis of Actin-based Endocytosis and Cytokinesis

20:10-20:50 **Dimitrios Vavylonis**, Lehigh University
Modeling Cdc42 Oscillations and Polarity Transition in Fission Yeast

20:50-21:30 **Ewa Paluch**, MPI for Molecular Cell Biology and Genetics
Polar acto-myosin contractility and cleavage furrow stability during cytokinesis

21:30-22:10 **John Cooper**, Washington University
Quantitative analysis of actin assembly and motility during endocytosis in living yeast cells

Thursday

7:00-8:30 Breakfast

8:30-9:10 **Gaudenz Danuser**, Harvard Medical School
Forces between Cells

9:10-9:50 **Dan Fletcher**, University of California Berkeley
Mechanical regulation of actin network assembly

9:50-10:30 **Mohammad Mofrad**, University of California Berkeley
Hydrodynamic interactions significantly alter the dynamics of actin networks

10:30-10:55 Coffee Break

10:55-11:35 **Fred MacKintosh**, Vrije Universiteit
Active fluctuations, mechanical integrity and self-organization in cytoskeletal networks

11:35-12:15 **Margaret Gardel**, University of Chicago
Mechanics of the Lamellar Actomyosin Cytoskeleton

12:15-13:30 Lunch

Free Afternoon

17:30-19:30 Dinner

19:30-20:10 **Martin Falcke**, Max Delbrück Center Berlin
A mechanism for actin based propulsion in morpho-dynamics, the force-velocity relation of fish keratocytes and reconstituted systems

20:10-20:50 **Leah Edelstein-Keshet**, University of British Columbia
Models of signaling pathways regulating eukaryotic cell motility

20:50-21:30 **Anders Carlsson**, Washington University
Spontaneous Waves and Patches in Dendritic Actin Nucleation

Friday

7:00-8:30 Breakfast

8:30-9:10 **Michael Kozlov**, Tel Aviv University
Physical model for self organization of actin cytoskeleton and adhesion complexes at the cell front

9:10-9:50 **Cécile Sykes**, Institut Curie
Biomimicking systems of cell shape changes

9:50-10:30 **Tatyana Svitkina**, University of Pennsylvania
Assembly of the contractile system in nonmuscle cells

11:30-13:30 Lunch – **Checkout by 12 noon.**

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ABSTRACTS

(in alphabetic order by speaker surname)

Anders Carlsson (Washington University)

Spontaneous Waves and Patches in Dendritic Actin Nucleation

Recent fluorescence imaging studies in Dictoystelium have shown that F-actin can spontaneously form traveling waves or moving patches at reduced actin concentrations. Possible mechanisms for such phenomena are investigated numerically using the "dendritic nucleation" model of actin network growth. The simulations treat actin network growth on a 3 by 3 micron piece of membrane. They store information about actin filaments subunit by subunit, giving an explicit three-dimensional picture of the actin network. The calculations include filament growth, capping, branching, severing, and random thermal motion. The dynamics of nucleation-promoting factors (NPFs) in the membrane are also included. The NPFs diffuse in the membrane, and detach in the presence of F-actin, thereby becoming inactivated. The simulations show three types of behavior: 1) traveling waves, 2) coherently moving patches, and 3) random fluctuations with occasional moving patches. Wave formation at low G-actin concentrations is favored by a long recovery time for NPFs which have been inactivated, and by weakness of the attractive interaction between filaments and the membrane. Raising the G-actin concentration results in a randomly varying distribution of F-actin. Lowering of the G-actin concentration below the optimal value for waves causes the waves to break up into patches. Similar effects are seen with decreasing and increasing capping-protein concentration, respectively.

John Condeelis (Albert Einstein College of Medicine)

Scaling and amplification of signals during tumor cell migration and dissemination in breast tumors

The actin binding protein cofilin is essential for directed cell migration and chemotaxis in tumor cells. In human and mouse breast tumors, activation of the cofilin activity pathway occurs in tumor cells during invasion, migration and dissemination. Part of this upregulation of activity results from overexpression of many genes in the cofilin activity pathway in metastatic tumor cells. Through its severing activity, cofilin increases the number of free barbed ends to initiate actin polymerization for actin-based protrusion in two distinct subcellular compartments in invasive tumor cells: lamellipodia and invadopodia. Cofilin severing activity is tightly regulated and multiple mechanisms are utilized to regulate cofilin activity. We have discovered that the primary on/off regulation of cofilin can be placed into two broad categories, both of which are important for inhibiting cofilin from binding to F-actin or G-actin: (1) Blocking cofilin activity by the binding of cofilin to either PI(4,5)P2 at lamellipodia, or cortactin at invadopodia. (2) Blocking cofilin's ability to bind to actin via serine

phosphorylation. Although the literature suggests that these cofilin regulatory mechanisms may be cell-type dependent, we propose the existence of a common cofilin activity cycle in which both operate and phosphoinositol / cortactin-mediated signaling plays a dominant role in amplification of signals from the EGFR during tumor cell chemotaxis and migration.

John A. Cooper (Washington University)

Quantitative analysis of actin assembly and motility during endocytosis in living yeast cells

Many kinds of cellular motility are driven, at least in part, by branched networks of growing actin filaments that push against a membrane. In the dendritic nucleation model, the Arp2/3 complex is central to the formation of these networks, binding to the side of an existing filament, creating a branch and nucleating a new filament. The proper regulation of Arp2/3 activity is likely to be critical for efficient generation of force and movement by actin networks. A number of Arp2/3 regulatory proteins have been identified. The model for their function is that they will bind to and / or activate the Arp2/3 complex via acidic motifs containing a conserved tryptophan residue. However, in vivo, testing of this model has been limited. In this study we have tested the hypothesis that the acidic domains of Arp2/3 regulators in the yeast actin patch will be critical for Arp2/3 recruitment and / or activation. We have used quantitative fluorescent microscopy to monitor the composition of the actin network in living cells harboring mutations that disrupt the interaction between Arp2/3 and its regulators. In contrast to prediction, we do not find a simple correlation between the defects in patch assembly and movement seen in these mutants and changes in the composition and dynamics of dendritic nucleation proteins within the patch. Taken together our data does not support the simple model that the primary role for Arp2/3 regulators is to recruit and activate Arp2/3. Rather our data suggests that these regulators may be playing more subtle roles in establishing functional networks in vivo.

Gaudenz Danuser (Harvard Medical School)

Forces between Cells

Tissue cells typically utilize their actomyosin contractile machinery to pull on their environment, which can be either the extracellular matrix or neighboring cells. The mechanical forces that a cell exerts and experiences have been shown to regulate fundamental cellular processes, including cell growth, proliferation, differentiation and migration. However, little is known about the spatial distribution of mechanical stress in tissues. In particular, the extent to which mechanical forces are communicated through cell-cell interactions across a tissue is not well understood.

We present a novel method, based on high resolution traction force microscopy, to measure mechanical stresses that are transmitted through cell-cell interfaces in small cellular clusters (~10 cells). Cells are classified according to their connection topology, i.e. the number of neighboring cells. We find that this parameter determines many of the mechanical properties in these islands, including the amount of force transmitted through a particular cellular interface. In order to determine how force balance in the cell cluster is locally achieved, we compared forces transmitted through cells to forces exerted on the underlying substrate. A correlation analysis of these forces reveals the length scale over which forces can be transmitted through the cell cluster. Furthermore, by molecular perturbations, we are identifying proteins that may be essential for long range mechanical communication in the cluster. The ability to quantify force communication between cells will allow us to examine

how cell-cell mechanical interactions contribute to overall tissue stress and vice versa. It will also allow us to investigate the role of mechanical stresses in establishing signaling gradients. This will further our understanding of the role of mechanical stress in processes that require fine coordination between cells, such as collective migration in morphogenesis and cancer.

Adriana Dawes (University of Alberta / Ohio State University)

Cortical thickening and negative curvature may help position the Par protein boundary in the early C. elegans embryo

Shortly after fertilization, single cell embryos segregate certain classes of proteins, Par proteins, to opposite ends of the cell. The boundary between these two domains is reliably positioned in wild type embryos, with little spatial variation. The mechanism responsible for positioning the protein boundary is not currently known, and in this talk I will discuss how thickening of the actin cortex and negative curvature may help position the Par protein boundary. This work is joint with David Iron (Dalhousie University).

Leah Edelstein-Keshet (UBC)

Models of signaling pathways regulating eukaryotic cell motility

I will highlight work done in my group on Rho GTPases (Cdc42, Rac, Rho) and phosphoinositides (PIs), their interactions, crosstalk, and effects on the cytoskeleton. I will discuss how both mathematical analysis (of simplified models) and larger scale simulations have helped us to understand parts of the mechanisms at play in eukaryotic cell polarization and motility. This work is joint with the following current and former group members: Nessy Tania, William Holmes, Ben Vanderlei, Stan Maree, Yoichiro Mori, Adriana Dawes, Alexandra Jilkine, Veronica Grieneisen, and Cory Simon

Martin Falcke (Max Delbrück Center for Molecular Medicine)

A mechanism for actin based propulsion in morpho-dynamics, the force-velocity relation of fish keratocytes and reconstituted systems

The morpho-dynamics of the lamellipodium leading edge have been shown to exhibit a few phenotypes, which are controlled by cell signaling (1). The variety of phenotypes reveals the internal dynamics of actin polymerization and retrograde flow inside the lamellipodium. Measurements of the force-velocity relation of motile fish keratocytes with an AFM cantilever represent another dynamic experiment allowing for drawing conclusions on the internal processes (2). Actin based motility has been reconstituted with ActA coated oil droplets (3) and ActA coated beads (4). Both systems exhibit steady and saltatory motion.

We present a mathematical model of actin based propulsion which provides a mechanism for velocity oscillations of Listeria (5), beads (6) and oil droplets (7). It also describes the mechanisms of the morpho-dynamic phenotypes (8) and the force velocity relation. The model accounts for the existence of two functionally different regions of the lamellipodium actin network observed in many studies (9). Network behavior is dominated by semi-flexible properties of filaments near the leading edge membrane, and it is more gel-like further towards the cell body (10). We include actin polymerization, filament binding to the leading edge membrane, retrograde flow, contraction of the actin network by myosin and a simple

description of cell adhesion to the substrate into the model.

1. Machacek, M., and G. Danuser. 2006. Biophys J 90:1439-1452.
2. Prass, M., K. Jacobson, A. Mogilner, and M. Radmacher. 2006. J. Cell Biol. 174:767-772.
3. Trichet, L., O. Campàs, C. Sykes, and J. Plastino. 2007. Biophysical Journal 92:1081-1089.
4. Bernheim-Groswasser, A., J. Prost, and C. Sykes. 2005. Biophys J 89:1411-1419.
5. Gholami, A., M. Falcke, and E. Frey. 2008. New Journal of Physics 10:033022.
6. Enculescu, M., and M. Falcke. 2011. New Journal of Physics 13:053040.
7. Enculescu, M., A. Gholami, and M. Falcke. 2008. Physical Review E 78:031915.
8. Enculescu, M., M. Sabouri-Ghomi, G. Danuser, and M. Falcke. 2010. Biophysical Journal 98:1571-1581.
9. Zimmermann, J., M. Enculescu, and M. Falcke. 2010. Physical Review E 82:051925.
10. Laurent, V. M., S. Kasas, A. Yersin, T. E. Schäffer, S. Catsicas, G. Dietler, A. B. Verkhovsky, and J.-J. Meister. 2005. Biophys J 89:667-675.

Dan Fletcher (UC Berkeley)

Mechanical regulation of actin network assembly

Actin filaments form organized networks that drive membrane protrusions, guide intracellular traffic, and govern mechanical rigidity of eukaryotic cells. In each of these roles, actin networks are exposed to a combination of external and internal forces that stretch, compress, and deform the filaments. While the biochemical basis for assembly and disassembly of the diverse actin network architectures found in cells has received significant attention, the role of physical inputs is less well understood. This talk will focus on optical and force microscopy studies that suggest that mechanical constraints play a crucial role in directing the assembly of actin networks.

Margaret Gardel (University of Chicago)

Mechanics of the Lamellar Actomyosin Cytoskeleton

The lamellar actomyosin cytoskeleton is essential for regulating cell shape and mechanical interactions with the surrounding extracellular environment. Diverse lamellar actin organizations ranging from contractile lamellar networks to stress fibers are observed in adherent cells. Although lamellar organization is thought to reflect the extent of cellular force generation, understanding of the physical behaviors of the lamellar actin cytoskeleton is lacking. To elucidate these properties, we visualized the actomyosin dynamics and organization in U2OS cells over a broad range of forces. At low forces, contractile lamellar networks predominate and force generation is strongly correlated to actomyosin retrograde flow dynamics with nominal change in organization. Lamellar networks build ~60% of cellular tension over rapid time scales. On rigid substrates, reorganization of the lamellar network into stress fibers results in moderate changes in cellular tension over slower time scales. On soft matrices, force generation by lamellar networks is unaffected, whereas tension-dependent stress fiber assembly is abrogated. Thus, organization of actomyosin into stress fibers does not have a pronounced effect on the ability of cells to generate tension on the ECM at focal adhesions. By contrast, stress fiber assembly at focal adhesions is required for compositional maturation of focal adhesions and extracellular matrix remodeling. These data elucidate the dynamic and structural signatures of the actomyosin cytoskeleton at different levels of tension and set a foundation for quantitative models of cell and tissue mechanics during cell adhesion, migration and division.

Melissa Gardner (University of Minnesota)

Microtubule Length Regulation by Depolymerizing Kinesins

Microtubules are dynamic filaments whose plus ends alternate between periods of slow growth and rapid shortening as they explore intracellular space and move intracellular organelles. A key question is how regulatory proteins such as the depolymerizing kinesins modulate catastrophe, the conversion from growth to shortening. To study this process, we reconstituted microtubule dynamics in the absence and presence of the kinesin-8 Kip3 and the kinesin-13 MCAK. We found that even in the absence of the kinesins, the microtubule catastrophe frequency depends on the length and age of the microtubule, indicating that catastrophe is a multistep process. Kip3 slowed microtubule growth in a length-dependent manner and increased the rate of accumulation of lattice destabilizing features that lead to catastrophe. In contrast, MCAK did not change the feature formation rate, but instead transformed catastrophe into a single step process. Thus, both kinesins are catastrophe factors, but influence microtubule length distribution in different ways: Kip3 mediates fine control of microtubule length by narrowing the distribution of maximum lengths prior to catastrophe, whereas MCAK promotes rapid restructuring of the microtubule cytoskeleton by making catastrophe a first-order random process.

Holly Goodson (University of Notre Dame)

Biological insights from computational modeling of microtubule dynamics

The microtubule (MT) cytoskeleton drives essential cell activities ranging from membrane transport to cell division. MT dynamic instability is fundamental to these processes, but the mechanisms of this behavior remain poorly understood. Broad consensus agrees that a structural cap related to the presence of GTP tubulin subunits promotes MT growth, but what constitutes a functional cap? What are the mechanisms of the random transitions between growth and depolymerization (“catastrophe” and “rescue”)? To address these questions, we have utilized a dimer-scale computational model of MT assembly. This model is based on present understanding of tubulin structure and biochemistry, explicitly considers both lateral and longitudinal bonds between subunits, and covers experimentally relevant spans of time (tens of minutes). It allows us to correlate macroscopic behaviors (dynamic instability parameters) with microscopic structures (tip conformations), and examine protofilament structure as the tip spontaneously progresses through both catastrophe and rescue. The model’s behavior suggests that several commonly-held assumptions about MT dynamics should be reconsidered. These include the ideas that the cap is a solid structure with defined edges (consistent with other recent models, the cap that emerges in our model is discontinuous), that GTP hydrolysis affects primarily lateral bonds (we find a requirement for an effect on longitudinal bonds), and that MTs grow as open sheets (instead of blunt sheets open at the seam, the model predicts tip extensions growing off of closed tubes). Most importantly, the model predicts that short interprotofilament "cracks" (laterally unbonded regions between protofilaments) exist even at the tips of growing MTs, and that rapid fluctuations in the depths of these cracks play a pivotal role in both catastrophe and rescue. The significance of the interprotofilament cracks suggests an obvious mechanism for the activity of MT binding proteins. We conclude that experimentally observed microtubule behavior can best be explained by a refinement of the “fluctuating cap” model first proposed by Chen and Hill. In this “stochastic cap” model, tubulin subunits hydrolyze GTP according to a first-order rate constant after they are incorporated into the lattice; catastrophe and rescue result from stochastic fluctuations in the number and distribution of the GTP subunits and the

extent of their lateral bonding. We are now analyzing the behavior of systems of these MTs to connect population level behaviors to MT dynamics, and to update classical concepts such as critical concentration.

Paul Janmey (University of Pennsylvania)

Driving cytoskeletal remodeling by extracellular matrix mechanics

Many cell types are sensitive to mechanical signals. Cells modulate proliferation, morphology, motility, and protein expression in response to substrate stiffness. Changing the elastic moduli of substrates alters the structure of focal adhesions, the formation of actin filament bundles, and the stability of intermediate filaments. The range of stiffness over which different primary cell types respond can vary over a wide range and generally reflects the elastic modulus of the tissue from which these cells were isolated. The molecular mechanisms by which cells detect substrate stiffness are largely uncharacterized, but simultaneous control of substrate stiffness and adhesive patterns suggests that stiffness sensing occurs on a length scale much larger than single molecular linkages and that the time needed for mechanosensing is on the order of a few seconds. Mechanosensing also depends on the type of adhesion receptor by which the cell binds, and therefore on the molecular composition of the specific extracellular matrix. Signalling from other transmembrane proteins such as hyaluronic acid receptors can in some cases provide crosstalk with integrin signalling to determine the stiffness-dependent phenotype. Several examples using different primary cell types show that the presence of hyaluronic acid in matrices that also contain ligands for integrins strongly affects the range of stiffness over which cells respond. As more examples of cell-type specific mechanosensing and response become documented, the variety and specificity of effects elicited by the mechanics of a cells environment continues to emerge as a critical determinant of cell and tissue function in vivo.

Chopra A, Tabdanov E, Patel H, Janmey PA, Kresh JY 2011. Cardiac myocyte remodeling mediated by N-cadherin-dependent mechanosensing. Am J Physiol Heart Circ Physiol 300: H1252-1266.
Tee, S. Y., J. Fu, C. S. Chen, and P. A. Janmey. 2011. Cell shape and substrate rigidity both regulate cell stiffness. Biophys J 100:L25-27

Kinneret Keren (Technion- Israel Institute of Technology)

Membrane tension in rapidly moving cells is determined by the balance between actin network assembly, adhesion and contraction

While the important role of membrane tension in cell motility is becoming apparent, little is known about how membrane tension is set and regulated. We measure membrane tension in rapidly moving fish epithelial keratocytes using a tether pulling assay. Membrane tension is found to range between 200-600 pN/μm in a population of keratocytes, values which are substantially higher than what has been measured for slower moving cells. To investigate the interplay between the motility machinery and membrane tension, we use various perturbations known to alter actin filament density, adhesion and contraction and study their effect. We find that decreasing the number of pushing filaments along the leading edge leads to a significant decrease in membrane tension. Moreover, we find that decreasing the adhesiveness of the substrate or increasing myosin-induced contraction also leads to lower membrane tension, whereas stronger adhesion or weaker contraction results in higher tension. These results suggest that tension is generated by growing actin filaments at the leading edge pushing against the inextensible membrane, and is relieved due to centripetal actin flow generated by myosin-powered contraction and mediated by the adhesion strength;

stronger actin pushing forces at the leading edge and/or a trailing edge which is harder to retract lead to higher tension. This notion is further supported by experiments in which large amounts of membrane are added to motile keratocytes, without changing their biochemical content, by fusion with giant unilamellar vesicles. In response to the significant increase in surface area (~25%) fused cells rapidly expand their lamellipodium, but continue to move with only minor changes in aspect ratio, speed and membrane tension. Together these results indicate that membrane tension is largely determined by the mechanical interplay between cytoskeletal forces and the membrane, and is not regulated through endo/exocytosis or other means, at least in rapidly and steadily moving cells such as keratocytes.

Michael M. Kozlov (Tel Aviv University)

Physical model for self organization of actin cytoskeleton and adhesion complexes at the cell front

Cell motion is driven by interplay between the actin cytoskeleton and the cell adhesions in the front part of the cell. The actin network segregates into lamellipodium and lamellum, whereas the adhesion complexes are characteristically distributed underneath the actin system. Here we suggest a physical model for this characteristic organization of the actin-adhesion system. The model is based on the ability of the adhesion complexes to sense mechanical forces, the stick-slip character of the interaction between the adhesions and the moving actin network, and a hypothetical propensity of the actin network to disintegrate upon sufficiently strong stretching stresses. We identify numerically three possible types of the system organization, all observed in living cells: two states in which the actin networks exhibits segregation into lamellipodium and lamellum whereas the cell edge either remains stationary or moves, and a state where the actin network does not undergo segregation. The model recovers the asynchronous fluctuations and outward bulging of the cell edge, and the dependence of the edge protrusion velocity on the rate of the nascent adhesion generation.

Herbert Levine (UCSD)

Eukaryotic Chemotaxis in Dictyostelium - Getting from the signal to the mechanics

Dictyostelium response to cAMP gradients is a well-studied model system for chemically guided amoeboid motion. One can think of this process as consisting of three stages: sensing the concentration field, deciding upon the motility response, and implementing the decision. This talk will review what has been learned about these stages in recent years, including some recent results comparing simple models of pseudopod dynamics with experimental data from cells chemotaxing in a microfluidics chamber. These models assume that the creation of new pseudopods is a random event which occurs with a probability distribution determined by a compass model which amplifies the external gradient. We show that commonly observed features of pseudopods, such as tip-splitting events and a tendency to alternate left-right protrusions, follow directly from such a model without the need for specific dynamical mechanisms.

Leslie M. Loew (University of Connecticut Health Center)

Modeling Actin Dynamics: Why the Details Matter

We have built a quantitative model for actin assembly based on the dendritic nucleation

mechanism using the Virtual Cell software system. The model starts with activation of Arp2/3 at the cell membrane by NWASP and incorporates several important actin binding proteins as well as consideration of the nucleotide-bound state of actin monomer and polymer. We first used this model to establish the steady state properties of the actin system in the absence of nucleation by Arp2/3. The model reproduces all the experimental behaviors derived from in vitro studies of sub-systems of the component molecules. We then constructed 3D spatial models of cells with thin lamellipodia and thick cell bodies. Activation of Arp2/3 in a small segment of lamellipodium membrane causes rapid localized actin nucleation and a buildup of F-actin to the 1mM level. The model provides an explanation for speckle microscopy experiments showing a remarkably sharp transition from filament assembly at the leading edge of cells to filament disassembly just 1μm away from the leading edge. We then applied the model to experimental data on SH3 domain signaling aggregates propelled by actin comet tails. Simulation results are in quantitative agreement with the observed profiles of actin distribution in the comets and show that the data requires unexpected stoichiometry in the upstream signaling molecules. The model was further extended to explain the results of Chemically Assisted Laser Inactivation (CALI) experiments on fluorescent capping protein, showing that these experiments could best be understood by accounting for the action of ENA/Vasp in the cell. Because this model and the simulation results are "open source", in the sense that they are publicly available and editable through the Virtual Cell database (http://vcell.org), they can be accessed, analyzed, modified and extended.

Fred MacKintosh (Vrije Universiteit)

Active fluctuations, mechanical integrity and self-organization in cytoskeletal networks

Much like the bones in our bodies, the *cytoskeleton* consisting of filamentous proteins largely determines the mechanical response and stability of cells. Unlike passive materials, however, living cells are kept far out of equilibrium by metabolic processes and energy-consuming *molecular motors* that generate forces to drive the machinery behind various cellular processes. We describe recent advances both in theoretical modelling of activity in cytoskeletal networks, as well as experiments on reconstituted in vitro acto-myosin networks and living cells. We show how force generation by myosin can lead to dramatic mechanical effects, including a strong stiffening of actin networks. We discuss how motor activity can stabilize floppy networks, and how this may be important for mechanical integrity of the cytoplasm. Furthermore, stochastic myosin activity can give rise to diffusive-like motion in elastic networks, which can be probed by microtubule fluctuations in living cells. We also study the self-organization of acto-myosin into contractile clusters through an active, multistage coarsening process. This can be understood in terms of the highly asymmetric load response of actin filaments: they can support large tensions, but they buckle easily under piconewton compressive loads.

Mohammad R. K. Mofrad (University of California Berkeley)

Hydrodynamic interactions significantly alter the dynamics of actin networks

To investigate actin dynamics we previously demonstrated that a rod model can reproduce dynamics of a single actin filament [1]. In a follow up study, we developed an averaged bead model to account for hydrodynamic interaction between multiple filaments. Our model reproduced the experimentally observed dynamics of actin filaments and furthermore it

predicted that the confinement increases persistence length [2]. This highlighted a significant error in studies that ignore hydrodynamic interaction effects in dense networks like the cytoskeleton. In this talk, I will present our new work to further explore and quantify the role of this error in different structural levels of the actin network. Our results suggest that hydrodynamic interactions between the actin network elements can alter the time scale of these movements by at least 2-20 folds in different levels of network structure. Our data suggest that for a single fiber, within the physiological range, hydrodynamic interaction effects between the cytoskeletal mesh-sized segments can significantly alter the dynamics of the cytoskeleton. Hydrodynamic interaction effects do not change the modal shapes of filament bending, but the relaxation times are underestimated without accounting for these effects. Exclusion of hydrodynamic interaction effects can result in significant (at least one order of magnitude) overestimation of shear loss modulus in an actin network. Taken together, our data suggest that it is critical to include hydrodynamic interaction effects to achieve accurate theoretical models of the cytoskeleton or to analyze experimental data.

[1] Chandran PL, Mofrad MRK (2009). Rods-on-string idealization captures semiflexible filament dynamics, PHYSICAL REVIEW E 79(1 Pt 1):011906.
[2] Chandran PL, Mofrad MRK (2010). Averaged Implicit Hydrodynamic Model of Semiflexible Filaments. PHYSICAL REVIEW E 81(3 Pt 1):031920.

Alex Mogilner (University of California at Davis)

Mechanical strategies for cell crawling

Recent joint experimental and theoretical studies elucidated two mechanical modules - 'actin treadmill in an unstretchable membrane bag' and 'graded viscoelastic actin-myosin contraction' - of cell motility. I will discuss computational results illustrating how these modules can be combined in cells migrating on 2D surfaces and through 3D matrices.

Inke Näthke (University of Dundee)

Early changes in tissue architecture in colorectal cancer

Changes in epithelial organisation accompany a range of human diseases from acute and chronic inflammatory conditions, bacterial infections, parasitic colonization and cancer. Understanding how epithelia are normally maintained and how molecular changes govern cellular and tissue changes in disease is necessary to develop effective treatments and detection methods. My research aims to understand the mechanisms that govern physiology of epithelial tissues, particularly gut epithelium, in health and disease.

The Adenomatous Polyposis Coli (APC) protein is a key regulator of normal epithelial architecture and physiology in the gut. This multifunctional protein has many binding partners that link it to signaling pathways that determine the properties and functions of epithelial cells and tissues. So we use the contributions of APC to gut tissue maintenance as a paradigm for understanding the earliest changes that occur in disease progression, particularly cancer.

Using high resolution imaging of whole tissue we have discovered novel architectural features of healthy and precancerous gut tissue. This includes continuous muscle-like structures that support intestinal tissue units and align with the outer muscle layer of the gut, changes in tissue shape that correlates with altered alignment of mitotic spindles specifically in stem cells, decreased cell migration, and corresponding accumulation of cells in a precancerous condition. I will discuss the implications of these changes but also how they

may relate to cell biological and biochemical changes associated with the mutations that produce them.

David J. Odde (University of Minnesota)

Rapid Microtubule Self-assembly Kinetics

Microtubule assembly is vital for many fundamental cellular processes. Current models for microtubule assembly kinetics assume that the subunit disassociation rate from a microtubule tip is independent of free subunit concentration. Using Total-Internal-Reflection-Fluorescence (TIRF) microscopy and a laser tweezers assay to measure in vitro microtubule assembly with nanometer resolution accuracy, we find that the subunit dissociation rate from a microtubule tip increases as the free subunit concentration increases. These data are consistent with a two-dimensional model for microtubule assembly, and are explained by a shift in microtubule tip structure from a relatively blunt shape at low free concentrations to relatively tapered at high free concentrations. Because both the association and the dissociation rates increase at higher free subunit concentrations, we find that the kinetics of microtubule assembly are an order-of-magnitude higher than currently estimated in the literature.

Karen Oegema (Ludwig Institute for Cancer Research/UCSD)

Using C. elegans embryo to dissect cell division mechanisms

Cytokinesis partitions the contents of the mother cell into the two daughter cells. In animal cells, cytokinesis is accomplished by constriction of a contractile ring, a filamentous network assembled from three types of protein polymers: actin filaments, bipolar filaments containing the motor protein myosin II, and membrane-associated septin filaments. The rate of contractile ring constriction is determined by the contractile stress within the ring and the viscous and elastic forces that oppose cortical deformation. Currently, we have little understanding of the mechanisms that generate contractile stress, and even less of the forces that oppose deformation. We are using the early *C. elegans* embryo as a model system to begin to address these questions. By analyzing the kinetics of contractile ring closure during the first five embryonic divisions in *C. elegans*, we have shown that contractile rings have an inherent ability to close at a constant overall rate, despite their decreasing size, during constriction. By monitoring sequential divisions during embryonic development and by analyzing cells in embryos of varying size, we could show that the constant rate at which a ring constricts is proportional to its initial perimeter. Larger rings constrict at a proportionally higher constant rate that is maintained throughout constriction. Consistent with the idea, originally proposed by Schroeder, that the contractile ring is a structure that consumes itself during constriction, we showed that the amount of three structural components of the ring (anillin, the septins and myosin II) decrease in proportion to perimeter. To explain these results we proposed a "contractile unit" model in which the contractile ring is assembled from units of fixed size that shorten at a constant rate without being lost. Larger rings would be composed of more units and would therefore constrict at a higher rate. In an attempt to understand what the units might be and what dictates constriction rate, we analyzed the effect of progressively depleting myosin II and the cytokinesis formin on cytokinesis. Although still in progress, initial results suggest that ring formation is more sensitive to myosin and formin levels than ring constriction. Interestingly, inhibition of myosin II and formin inhibit different steps during cleavage furrow formation.

Ewa Paluch (MPI-CBG, Dresden and IIMCB, Warsaw)

Polar acto-myosin contractility and cleavage furrow stability during cytokinesis

Cytokinesis relies on tight regulation of the mechanical properties of the cell cortex, a thin acto-myosin network lying under the plasma membrane. Although most studies of cytokinetic mechanics focus on force generation at the equatorial acto-myosin ring, a contractile cortex remains at the poles of dividing cells throughout cytokinesis. Whether polar forces influence cytokinetic cell shape is poorly understood. Combining cell biology and biophysics, we demonstrate that the polar cortex makes cytokinesis inherently unstable and that any imbalance in contractile forces between the poles compromises furrow positioning. We show that limited asymmetric polar contractions occur during normal cytokinesis, and that perturbing the polar cortex leads to cell shape oscillations and division failure. A theoretical model based on a competition between cortex turnover and contraction dynamics accurately accounts for the oscillations. We further propose that blebs, membrane protrusions that commonly form at the poles of dividing cells, stabilize the position of the cleavage furrow by acting as valves releasing cortical contractility. Taken together, our findings show that the physical properties of the entire cell are integrated into a fine-tuned mechanical system ensuring successful cytokinesis.

Thomas D. Pollard (Yale University)

Quantitative Analysis of Actin-based Endocytosis and Cytokinesis

We combine structure determination, biophysical measurements on proteins and cells with mathematical modeling to understand how fission yeast cells assemble actin filaments for clathrin-mediated endocytosis and cytokinesis.

Arp2/3 complex mediates the assembly of branched actin filaments at sites of clathrin-mediated endocytosis. Kinetic and thermodynamic analysis, crystal structures and EM reconstructions have defined a surprisingly complex pathway of branch formation. Using fluorescent fusion proteins we counted the numbers of >20 proteins during the time course of assembly and disassembly of endocytic actin patches. These numbers constrain simulations of a deterministic mathematical model of the process. A mechanism based on the dendritic nucleation hypothesis is robust and simulations correctly predicted some missing biochemical information. The rapid loss of actin filaments from patches involves diffusion away of short filament fragments in addition to depolymerization from the ends.

Cells assemble a cytokinetic contractile ring from about 65 macromolecular complexes called nodes located around the equator. At the transition into anaphase formin Cdc12p joins nodes where it nucleates and elongates actin filaments. Myosin-II motors in nearby nodes capture growing filaments and use transient interactions to pull nodes into a contractile ring. Monte Carlo simulations of a mathematical model of these reactions using parameter values measured in live cells reliably produce anatomically realistic contractile rings in the same time as live cells (Vavylonis et al. Science, 2007) and correctly predict the outcomes of experimental alterations of the reactions such as changing the rate that cofilin severs actin filaments. After maturing for 25 min through the addition of other proteins, the contractile ring constricts to pinch the cell in two. Simulations of a mathematical model by our collaborators Matthew Stachowiak and Ben O'Shaughnessy (submitted for publication) reproduce the time course of ring constriction that we observe in protoplasts without cell walls.

Jennifer Ross (University of Massachusetts Amherst)

Controlling Microtubules Through Severing

Regulation of microtubule dynamics, length, and location is essential for cell morphology, division, and migration. Microtubule-severing enzymes are ATPases that are known to remodel microtubule arrays during interphase and mitosis, in flagella and axons. Microtubule-severing enzymes remove tubulin dimers from the middle of the microtubule to cut the filament; thus, they are lattice destabilizers. We are interested in the inherent biophysical activities of these proteins, and focus on two families: katanin and fidgetin. We use two-color single molecule total internal reflection fluorescence imaging to visualize purified severing enzymes and microtubules *in vitro*. We find that katanin localizes to locations of severing activity including the microtubule ends to cause selective removal of terminal dimers, appearing as depolymerization. Katanin also binds to and severs at locations of lattice defects. Like katanin, we find that fidgetin can sever and depolymerize microtubules *in vitro*, but fidgetin's localization and activities are different from katanin.

David Sept (University of Michigan)

Microtubule mechanics at varying length scales

The mechanics of the actin filaments and microtubules are key to many of their cellular functions. These polymers have been extensively studied using a wide range of biophysical techniques, and we have sought to connect the dynamics we observe in all-atom molecular dynamics simulations with continuum mechanics properties. We have developed coarse-graining techniques that allow us calculate mechanical properties of these polymers using coarse-grained molecular dynamics as well as more mesoscopic descriptions. Our findings match very well with experimental measurements and allow us to probe how the atomic level effects of small molecules and/or point mutations manifest themselves at the level of the polymer.

Sean Sun (Johns Hopkins University)

Cytoskeletal dynamics and mechanosensation of adherent cells

Using a mechnochemical model of biological friction, and analytic estimates based on this model, we propose a simple picture of cytoskeletal re-organization during cell adhesion movement. We show that this model predicts formation of stress-fibers and is substrate stiffness dependent. Larger bundles of actin appear preferentially on stiff substrates. The model provides a mechanical basis for cellular mechanosensation. When coupled with regulatory chemical networks for myosin activity, the model can be a starting point for understanding long term stiffness sensing in tissue cells.

Tatyana Svitkina (University of Pennsylvania)

Assembly of the contractile system in nonmuscle cells

The contractile system of nonmuscle cells consists of interconnected actomyosin networks and bundles anchored to focal adhesions. The initiation of the contractile system assembly is poorly understood structurally and mechanistically, whereas system’s maturation heavily depends on nonmuscle myosin II (NMII). Using platinum replica electron microscopy in combination with fluorescence microscopy, we characterized the structural mechanisms of

the contractile system assembly and roles of NMII at early stages of this process. We show that inhibition of NMII by a specific inhibitor, blebbistatin, causes disassembly of bipolar NMII filaments, peripheral accumulation of unpolymerized activated NMII, transformation of lamellipodia into unattached ruffles, and loss of focal complexes, the precursors of mature focal adhesions, in addition to complete disassembly of mature stress fibers and focal adhesions. De novo assembly of the contractile system after blebbistatin washout begins with quick and coordinated recovery of lamellipodia and focal complexes that precedes the appearance of NMII bipolar filaments, suggesting that unpolymerized NMII activated at the cell periphery promotes formation of focal complexes and lamellipodia. Filopodial bundles and concave actin bundles formed by filopodial roots at the lamellipodial base are preferred sites for initial formation of focal complexes and subsequent assembly of NMII filaments, suggesting a load-dependent mechanism of NMII polymerization along anchored bundles. Over time, accumulating NMII filaments transform the precursor structures, focal complexes and associated bundles, into stress fibers and mature focal adhesions. However, semi-sarcomeric organization of stress fibers develops at much slower rate. We discuss potential mechanisms of NMII-dependent initiation of the contractile system assembly.

Cécile Sykes (Institut Curie / CNRS / Université Paris)

Biomimicking systems of cell shape changes

In order to unveil generic mechanisms of cell movements, we designed stripped-down experimental systems that reproduce cellular behaviours in simplified conditions. We have reconstituted the actin cortex of cells inside liposomes, and used it as a simplified system to study cell mechanics and endocytosis. Such stripped-down systems allow to address biological issues within a controlled, simplified environment.

We will present a characterization of reconstituted actin cortices inside liposomes, their mechanical properties measured by tube pulling, and liposome spreading, as done previously in cells. Moreover, we will show that endocytosis can be mimicked with actin and membranes in the case of Shiga toxin entry.

Jay Tang (Brown University)

A multiple spring model that predicts bipedal locomotion of crawling cells

The locomotion of crawling cells can be characterized by a variety of cell shape dynamics ranging from complex ruffling and bubbling to oscillatory protrusion and retraction. Shape changes are especially prevalent in fast moving fish keratocytes where periodic sticking and slipping at opposite sides of the cell's broad trailing edge generate bipedal locomotion and lateral motion of the nucleus. We use a two model of coupled springs to study the mechanical coupling and adhesion force that recapitulate the dynamics of these crawling cells. The model consists of point-like cell elements representing the fundamental regions of shape kinematics: leading edge, opposite sides of the trailing edge, and cell nucleus. Each component, other than the driving leading edge, can either stick or slip dependent on the local velocity. Based on simple assumptions about cell physiology, such as cell symmetry and nucleus confinement to the central region of the cell, we determine there to be only four physiologically feasible permutations of mechanical couplings between elements. We compare and contrast these four configurations and find that centralized mechanical coupling to the cell nucleus holds the key to generating both realistic bipedal shape dynamics and nuclear lateral motion. The configuration we propose therefore captures the essential

feature of nuclear motion not addressed previously.

Arpita Upadhyaya (University of Maryland)

Spreading itself thin: Biophysics of cell junctions in the immune system

Spreading of cells involves large scale physical rearrangements of the actin cytoskeleton and cell membrane. The spreading of T and B-lymphocytes on antibody coated substrates mimics the formation of the immune synapse, a multi-protein signaling machine. As signaling events are initiated within a minute of contact, early spreading leading to rapid increases in contact area and accumulation of receptors is a critical step of the immune response. The dynamics of the membrane and cytoskeleton during formation of contact and their effect on signaling is not well understood. We have studied the kinetics of T cell spreading, the morphology of the membrane, actin dynamics and simultaneously the spatiotemporal localization of signaling clusters in the very early stages of contact formation. We observed two modes of spreading, characterized by dramatic differences in membrane topography and cytoskeletal dynamics. Formation of signaling clusters was closely correlated with the movement and topography of the membrane in contact with the activating surface. Our observations suggest a possible link between the signaling function of cells and the physical nature of the cell membrane. We also observed membrane waves driven by actin polymerization at the cell substrate interface. Membrane deformations induced by such wavelike organization of the cytoskeleton may be a general phenomenon that underlies cell movement and cell-substrate interactions. Finally, we studied T cell spreading on elastic substrates to investigate the possible roles of substrate rigidity on spreading and signaling activation.

Dimitrios Vavylonis (Lehigh University)

Modeling Cdc42 Oscillations and Polarity Transition in Fission Yeast

Cells regulate states of polarization by activating and inactivating cortical Rho GTPases at cell growth sites. In collaboration with M. Das and F. Verde at U. Miami, we examined GTPase Cdc42 in the model organism fission yeast that undergoes a monopolar to bipolar growth transition ("new-end take-off"). We found that tip-bound active Cdc42 concentrations correlate with changes in growth pattern and exhibit large oscillations with an average period of five minutes. We developed a mathematical model of this process invoking Cdc42 autocatalytic amplification, competition between growth sites, and delayed negative feedback. This model restricts short cells to monopolar states while allowing longer cells to be bipolar. Linear stability analysis and numerical methods identify stable fixed points over a twofold increase in cell length. The model reproduces the observed oscillatory dynamics and survived several experimental tests. These findings indicate that Cdc42 oscillations facilitate transitions between polarization states and are critical for active redistribution of growth.

Alexander B. Verkhovsky (École Polytechnique Fédérale de Lausanne)

Interplay between cytoskeletal forces, membrane tension, and hydrostatic pressure in rapidly migrating cells

In motile cells, membrane pressure at the membrane/cytoplasm interface is balanced by the cytoskeletal forces and hydrostatic pressure. In particular, it is believed that protrusion of the lamellipodia is controlled by the balance of membrane pressure and the pressure generated

by actin polymerization, while bleb formation results from the balance of membrane pressure and hydrostatic pressure. However, exact contributions of these forces to the cell shape and motion are not known. Membrane pressure is a product of tension and local curvature of the membrane; thus, to know the membrane pressure one has to know the exact three-dimensional shape of the cell. We have developed a simple approach to measure cell vertical profile and volume, which is based on the displacement of the fluorescent cell-impermeable dye dissolved in the medium. To get insight into the balance of forces at the membrane interface, we measure membrane tension (with tether assay), protrusion velocity, vertical profile, and volume dynamics in migrating fish epidermal keratocytes subjected to cytoskeletal drugs and volume perturbations. Inhibiting myosin-dependent contraction with blebbistatin resulted in irregular cell shape, and reduced protrusion velocity and membrane tension (variable and low tether forces as compared to a force distribution centered around 35-40 pN in control cells). Remarkably, hypoosmotic treatment normalized blebbistatin-treated cells by restoring their tension, protrusion velocity, and shape, and also increased tension and protrusion velocity in control cells. In general, treatments inducing increase in membrane tension and hydrostatic pressure also increased cell velocity, while treatments decreasing membrane tension decreased cell velocity. Vertical profile measurements showed that hypoosmotic treatment induced persistent cell swelling and substantial increase in the height of the lamellipodia. We propose that hydrostatic pressure that could be generated due to either myosin-dependent contraction or osmotic gradient helps to maintains optimal vertical profile of the lamellipodium and thus cooperates with actin-dependent protrusion. Supported by Swiss National Science Foundation, Swiss SystemsX and NCCBI.

Orion Weiner (UCSF)

Mechanical tension spatially restricts signals to the leading edge during neutrophil migration

While no one knows how neutrophils and other cells establish a single zone of actin assembly during migration, many assume that the leading edge prevents formation of additional fronts by generating long-range diffusible inhibitors or by sequestering essential polarity components. Both mechanisms require rapid diffusion of signaling components through the cytosol. We have used morphological perturbations, cell severing experiments, and computational simulations to show that diffusive-based mechanisms are not sufficient to explain how the pseudopod inhibits protrusions elsewhere. Tension could serve as a long-range inhibitor under our experimental conditions. We find that membrane tension approximately doubles during leading edge protrusion, and experimentally increasing tension via micropipette aspiration rapidly inhibits protrusions, actin assembly, and Rac activation. Thus, protrusion-based increases in tension, rather than diffusible molecules generated or sequestered at the leading edge, exert long-range inhibition that constrains the spread of the existing front and prevents the formation of secondary fronts.

Denis Wirtz (Johns Hopkins University)

Cell Motility in 3D

Two-dimensional (2D) *in vitro* culture systems have for a number of years provided a controlled and versatile environment for the study of cell adhesion and migration, two interrelated cell functions critical to cancer metastasis. However, the organization and functions of focal adhesion proteins in cells embedded in physiologically more relevant 3D matrices is qualitatively and functionally different from their organization and functions on

conventional 2D planar substrates. In a 3D, crosslinked, fibrillar collagen matrix, cell migration and protrusion activity are still regulated by focal adhesion proteins, such as p130Cas, FAK, Zyxin, Vinculin, Talin, and VASP, but differently from the 2D case. This talk will describe the implications of the dependence of focal adhesion protein-based cellular functions on microenvironmental dimensionality in cancer. We will discuss the implications of this work in cancer metastasis.

Jennifer Zallen (Sloan-Kettering Institute)

Shaping the embryo: Cellular dynamics in development

A major challenge in developmental biology is to understand how large-scale changes in tissue structure are generated on a cellular and molecular level. In Drosophila, polarized cell movements cause the embryo to double in length from head to tail and narrow in width from back to front, creating the basic layout of the body plan. This process is characterized by a striking directionality in which large populations of cells align their movement along a common axis. We found that proteins involved in cell adhesion and contractile force generation are asymmetrically localized in intercalating cells, where they participate directly in polarized cell behavior. A polarized contractile network could provide the global spatial cue that guides cell movement, while differential adhesion may regulate dynamic interactions between cells. To understand the cell behaviors that lead to axis elongation, we carried out live imaging studies and found that intercalating cells organize into multicellular rosette structures that form and resolve in a strictly directional fashion. Rosette formation occurs through a mechanical feedback loop in which myosin generates tension and tension recruits additional myosin to the cortex, triggering a wave of actomyosin contractility that increases the number of cells engaged in intercalary behavior, promoting efficient elongation. Rosette behaviors have been shown to occur in vertebrates and may represent a general mechanism linking single-cell asymmetries to global tissue reorganization. To understand how genes encode the forces that generate tissue structure, we are currently performing large-scale genetic screens to identify the genes that are required for axis elongation and developing computational approaches to analyze multicellular dynamics in three dimensions.