and early genes are upregulated even at the lowest TNF-α concentrations, indicating digital signaling to gene expression. Late gene expression requires persistent NF-κB activity that is induced only at highest signal levels. The measurements reveal the activation threshold, a hypersensitive dynamic range and saturation, and shows that as few as two bound receptors can activate the pathway. The cells further encode TNF-α concentration information by modulating the temporal dynamics of NF-κB, with higher concentrations resulting in faster activation and more oscillations. Our results -in addition to their biological significance- highlight the importance of high-quality, high-throughput measurements at the single-cell level in understanding how biological systems operate.

2220-Plat

Quantitative Test for Mirror Symmetry Relationship between Sister Cells

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Cell shape determination is a dynamical process modulated by input from genetic and signaling pathways. In order to understand cell morphology as a dynamical system it is critical to determine the degree of spatial inheritance, that is, the degree of influence of spatial organization within a mother cell on the organization of her daughter cells. Visual comparison of symmetry relations between daughters was used by Albrecht-Buehler to probe spatial inheritance in a series of papers published in the 1970's. His results, that sister cells are sometimes mirror images of each other, were interpreted as reflecting spatial inheritance during cell division. We have reinvestigated these claims using quantitative image analysis and several different shape-comparison algorithms to test the symmetry relations between sister cells. Applying these methods to fixed and live RPE-1 and NIH 3T3 cells, we obtained the following results: (A) sister cells are quantitatively more similar in shape than pairs of unrelated cells, (B) When sister cells show a significant degree of shape similarity, they tend to be related by mirror symmetry, (C) the shape similarity between sister cells is highest soon after division and decays on a time scale of several hours, (D) the set-theoretic union of the two sister cell shapes is related to the shape of the mother with a degree of similarity that decays as a function of time before and after division. We have also developed methodologies to compare similarity of internal actin stress fiber organization between sisters and to compare the migration trajectories of sister cells as they move away from the site of division.

2221-Plat

Tuning the Range and Stability of Multiple Phenotypic States with Coupled Positive-Negative Feedback Loops

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The ability of a cell population to maintain simultaneously multiple phenotypic states is crucial for cell fitness. It is well known that the presence of a positive feedback is sufficient to generate multistability. However, many transcription networks contain counteracting positive and negative feedbacks, which indicates that this multiloop design provides additional advantages. Through an experimental and computational study in an interlinked positive and negative feedback motif in which each feedback strength is modulate independently, we explore the dynamic properties of this interlinked network. Our results show that the transition rates between different phenotypic states are not modulated by the positive, but only by the negative feedback strength. These results indicate that the negative feedback strength determines the phenotypic behavior of the cell populations, modulating the range and stability of different multistable states. By measuring noise levels in mRNA expression we show that this differential behavior is possible because noise levels are modulated independently by each feedback motif inside this genetic network. This suggests that in this coupled design, the strength of the negative feedback loop may be tuned to allow a population to enhance its fitness by changing the rate of stochastic transitions between different states, thus ensuring that cells may be prepared to confront a given level of environmental fluctuations.

2222-Plat

The Effects of Spatial Heterogeneity and in vivo Crowding on the Lac Genetic Circuit

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Standard deterministic and stochastic models used to explore the dynamics of cellular biochemical networks typically ignore spatial degrees of freedom (by assuming the cell is well-stirred). Spatial heterogeneity has been neglected due to the lack of both data regarding cellular localization and computational methodologies to simulate such models. Advances in *in vivo* imaging techniques, including cryo-electron tomography and single-molecule fluorescence microscopy, have begun to reveal the organization and dynamics of biomolecules inside the cell. Likewise, graphics processing units (GPUs) now provide the computational power to perform three-dimensional simulations of cell-scale models. Here, the effects of incorporating spatial information and molecular crowding into a stochastic model of the lactose utilization genetic circuit are reported. We use our recently developed lattice-based Monte Carlo simulation technique [1] to sample the reaction-diffusion master equation describing the lac circuit in an *Escherichia coli* cell. Parameters are obtained from published *in vivo* single molecule studies. By comparing to the well-stirred model, it is shown that spatial degrees of freedom introduce a source of noise into the circuit. Such spatial noise is a component of the extrinsic noise of a genetic system and we put bounds on its contribution. In certain fluctuating environments, spatial noise is found to influence the switching properties of the circuit leading to population distributions that cannot be predicted using well-stirred models. Finally, the model suggests new single molecule experiments to probe the lac circuit and provides estimates of the spatial and temporal resolution required. The integration of lattice microbe models with systems biology descriptions of cellular networks is also discussed.

[1] Roberts, Stone, Sepulveda, Hwu, and Luthey-Schulten, ''Long time-scale simulations of *in vivo* diffusion using GPU hardware'', In *The Eighth IEEE International Workshop on High-Performance Computational Biology* (2009).

2223-Plat

Macroscopic Kinetic Effect of Cell-To-Cell Variation in Biochemical Reactions

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Genetically identical cells under the same environmental conditions can show strong variations in molecular content such as in protein copy numbers due to inherent stochastic events in individual cells. We here develop a theoretical framework to address how variations in enzyme abundance affect the collective kinetics of metabolic reactions observed with a population of cells. Kinetic parameters measured at the cell population level are shown to be systematically deviated from those of single cells, even within populations of homogeneous parameters. The Michaelis-Menten kinetics, besides, can be destroyed at such population level. Our findings elucidate the novel origin of discrepancy between *in vivo* and *in vitro* kinetics, and offer the potential utility of single-cell metabolomic analysis.

2224-Plat

On Population Heterogeneity and Coexistence of Bacteria and Phage

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Population dynamics experiments using bacterium Escherichia coli and phage lambda are carried out in a well-mixed environment. In all trials, the long-term behavior is the coexistence of the bacterial and the phage populations. This outcome is remarkably insensitive to various experimental conditions such as the initial bacterial population size and the initial infection ratio. Upon isolation of survival bactaria and regrown in a phage-free environment, the newly generated bacteria exhibit phage sensitivity similar to their parental cells. Thus the coexistence of bacteraium and phage is not due to genetic mutation but is intrinsic to nonlinear dynamics of the interacting species. Our measurements showed that the bacterial population is sustained by a small number of persistors that provide ''physiological refuge'' against phage infection. The measured bacterium and phage population dynamics can be reasonably account for by a simple mathematic model that takes into account stochastic switching between normal cells and persister cells.

2225-Plat

Driving DNA Tweezers with an in vitro Transcriptional Oscillator

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In recent years, a considerable number of DNA-based molecular devices have been developed whose motion can be controlled by nucleic acid ''effector'' strands. For instance, the paradigmatic DNA ''tweezers'' system consists of two double-stranded arms connected by a flexible single-stranded ''hinge'' that can be closed or opened by the addition of so-called ''fuel'' strands. We have recently shown that this motion can also be driven with RNA rather than with DNA effectors. In order to realize an autonomously running biochemical system, we now utilized an artificial gene regulatory circuit in vitro to control the temporal behavior of the DNA tweezers. The gene circuit is a minimalistic feedback system that contains two genes from which regulatory RNA molecules are transcribed. The regulators mutually influence their production in an activatory and inhibitory manner, respectively,resulting in oscillatory network dynamics. We experimentally demonstrate how this transcriptional oscillator can be used to ''clock'' the motion of the DNA nanodevice in a variety of different ways. Furthermore, we investigate the robustness of the oscillator system with respect to increasing ''load'', i.e., tweezers concentrations.

Minisymposium 3: Tug of War: Molecular Motor Interactions

2226-MiniSymp

Opposite-Polarity Motors Activate One Another to Trigger Cargo Transport in Live Cells

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Intracellular transport, unlike in vitro, is typically bi-directional – consisting of a series of back and forth movements. Kinesin-1 and cytoplasmic dynein require each other for bi-directional transport of intracellular cargo along microtubules i.e. inhibition or depletion of kinesin-1 abolishes dynein-driven cargo transport, and vice versa. Using Drosophila S2 cells, we demonstrate that replacement of endogenous kinesin-1 or dynein with an unrelated motor of the same directionality, and targeted to peroxisomes, activates peroxisome transport in the opposite direction. However motility-deficient versions of motors, that retain the ability to bind microtubules and hydrolyze ATP, do not activate peroxisome motility. Thus any pair of opposite-polarity motors, provided they move along microtubules, can activate one another. These results demonstrate that mechanical interactions between opposite-polarity motors are necessary and sufficient for bi-directional organelle transport in live cells.

2227-MiniSymp

Motor Number Controls Cargo Switching at Actin-Microtubule Intersections *in vitro*

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Cellular activities such as endocytosis and secretion require that cargos switch between the microtubule (MT) and actin filament (AF) networks. Previous evidence suggests that switching may be regulated through a tug-of-war between MT and AF motors. To test the hypothesis that motor number can be used to direct the outcome of this tug-of-war, we reconstituted cargo switching at MT-AF intersections in a minimal system. We attached varying numbers of myosin-V and dynein-dynactin molecules to polystyrene beads and used an optical trap to position these beads near MT-AF intersections. Beads displayed a median pause time of 9 s at the intersection before exiting on a track. At least 23% of beads underwent rotation at intersections suggesting that competing motors apply a torque on their cargo. Force measurements to quantify the number of actively engaged motors show that stall force scales with the number of myosin-V motors as has previously been shown for kinesin-1 and dynein. Largely independent of whether it enters the intersection on the MT or AF, a bead with a myosin-V:dynein-dynactin force ratio of 0.5 (1 myosin-V to 4 dynein-dynactins) has a $>$85% probability of exiting on the MT. A bead with a myosin-V:dynein-dynactin force ratio of 1 (1 myosin-V to 2 dynein-dynactins) has an approximately equal probability of exiting on the MT, exiting on the AF, or remaining at the intersection. A bead with a myosin-V:dynein-dynactin force ratio of 4 (2 myosin-Vs to 1 dynein-dynactin) has a $>$95% probability of exiting on the AF. We have developed a statistical model that delineates the relationship between switch probability and motor number. Thus, cargo switching can be tuned via combinations of 1-4 myosin-V and dynein-dynactin motors through a simple force-mediated mechanism. Supported by P01 GM087253.

2228-MiniSymp

Myosin Va and Myosin VI Engage in a "tug of war" on Actin Tracks While Transporting Cargoes *in vitro*

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Myosin Va (myoVa) and myosin VI (myoVI) are processive molecular motors that transport cargo in opposite directions on actin tracks. Since myoVa and myoVI may colocalize to the same cargo *in vivo*, these motors may undergo a tug of war. Therefore, we sought to characterize the stepping dynamics of single myoVa and myoVI motors *in vitro* as they mechanically interact when linked together by a Qdot cargo. Expressed myoVa-HMM with an N-terminal biotin tag were labeled with streptavidin-Qdots (565nm) while expressed dimerized myoVI-HMM were Qdot(655nm)-labeled on an exchanged calmodulin. The effective tug of war on actin filament tracks (25mM KCl, 2mM ATP, 22°C) was observed in TIRF with 6nm resolution, allowing individual steps to be detected. MyoVa won ~80% of the time and regardless of which motor won, its stepping rate was reduced ~50% below its unloaded value due to the resistive load of the opposing motor. Interestingly, as the winning motor stepped forwards (myoVa, 73nm; myoVI, 56nm) the opposing motor stepped backwards (myoVa, 68nm; myoVI, 65nm) at the same rate, although myoVI appeared to be dragged at times. Why does myoVa dominate when its stall force is similar to myoVI? Given the probability that both myoVa and myoVI take occasional backsteps and experience a 2-3-fold reduction in stepping rate when winning, we estimate based on optical trapping data (Altman et al., 2004; Kad et al., 2008) that myoVa exerts a 50% greater resistive load compared to myoVI, providing a potential advantage to myoVa. Differences in the length of the myoVa and myoVI constructs could lead to each motor experiencing different vectorial force components, the potential that this may influence the outcome of the tug of war is being investigated.

2229-MiniSymp

Collective Behavior of Antagonistically Acting Kinesin-1 Motors

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Active cellular transport along microtubules is driven by the concerted operation of molecular motors. This often leads to complex dynamic behaviors such as stop-and-go or bidirectional movements. An important situation arises when motors act antagonistically in a tug-of-war scenario. In order to mimic the action of antagonistic motors, we performed gliding motility assays of antiparallel microtubule doublets driven by kinesin-1. In this configuration the lengths of the individual microtubules of the doublet determined the numbers of motors available to act against each other. At high motor density, we found two possible modes of movement: slow movements, where the doublets were almost stalled, and fast movements, where the doublet velocity was close to the velocity of single microtubules. Moreover, we observed a range of microtubule length differences where both modes coexisted. We developed a theoretical description that quantitatively describes the experimental data. In order to account for the two modes of movement, as well as for the possibility of their coexistence, it was necessary to take into account (i) the finite stiffness of the linkers by which the motors are connected to the substrate, (ii) the load-dependence of the detachment rate of single motors, and (iii) a non-linear force-velocity relationship of single motors. Our results show that mechanical interactions between motors can generate coexisting transport regimes with distinct velocities.

2230-MiniSymp

Iinteractions between Motor Proteins can Explain Collective Transport of Kinesins

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The collective function of multiple motor proteins is central to a variety of transport processes in cells. Yet, how key transport parameters depend on motor number and inter-motor interactions remains unclear. Recent experiments[1] have allowed the dynamic properties of two coupled kinesin-1 molecules to be examined using 'single-molecule' biophysical techniques. These studies have revealed that negative motor cooperativity plays a significant role in collective kinesin dynamics. Current theoretical models that neglect intermolecular interactions cannot capture this behavior. We propose a new theoretical approach, based on discrete-state stochastic models, which allows us to describe complex aspects of coupled kinesin dynamics. By treating intermotor interactions explicitly, these models can be used to reconcile important differences between predictions based on non-cooperative (additive) behaviors, and observations of negative kinesin cooperativity.