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Heat Shock Proteins Regulate Structure of Intermediate Filament Networks

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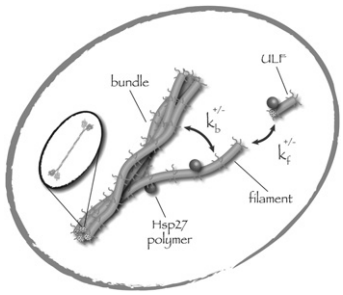
Living cells exhibit an enormous bandwidth of mechanical and morphological properties. These are mainly determined by the cytoskeleton, a composite network constituted of three classes of biopolymers: actin filaments, microtubules and intermediate filaments. While the interaction of actin filaments is mediated by a variety of cross-linking proteins, keratin intermediate filaments exhibit an inherent tendency to interact with each other under physiological salt conditions. Here we show, how small heat shock proteins can modulate such interactions in reconstituted keratin networks resulting in a drastic alteration of network structure and morphology. This mechanism provides an essential tool for cells to regulate the organization of their intermediate filament cytoskeleton.



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Sperm Cell Crawling in the Nematode Caenorhabditis Elegans

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Cell motility is important in biological processes, such as the immune response and cancer cell metastasis. Actin is implicated in most amoeboid cell movement, but *Caenorhabditis elegans* sperm cells lack actin, and their motility is driven by the Major Sperm Protein (MSP) cytoskeleton. MSP and actin form filament systems that are functionally similar, and the sperm cell therefore represents a simplified cellular model for studying the general properties of biopolymer-based movement.

We use the sperm cell to test how membrane tension affects movement and cytokeleton dynamics. We relax or tense the cell membrane with biochemical treatments or osmotic shock and we observe that membrane tension reduction is correlated with a decrease in cell displacement speed, whereas an increase in membrane tension enhances motility. We show evidence for the idea that membrane tension optimizes motility by streamlining polymerization in the direction of movement, thus adding a layer of complexity to our current understanding of how membrane tension enters into the motility equation.

We also use the sperm cell to study the effect of adhesion on retrograde flow and movement. We prepare substrates of varying compositions, and observe the interplay between cytoskeletal flows and the efficiency of displacement.

In the long term, study of the simplified MSP system and comparison to actin based movement should lead to a better understanding of the fundamental principles cell motility.

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Electron Tomography of MSP Filaments Derived from the Amoeboid Sperm of Ascaris Suum

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Locomotion of nematode sperm is remarkably similar to that of most other crawling cells but is powered by a system of filaments composed of major sperm protein (MSP) instead of the actin-myosin machinery typically associated with amoeboid motility. The MSP motility apparatus has been reconstituted *in vitro*, and individual MSP filaments can be generated by the addition of ATP to detergent-treated *Ascaris* sperm cytosol. Filaments formed in this way have been examined using both electron cryo-tomography and also conventional tomography of negative-stained samples. This has allowed for the structural analysis of filaments formed in the presence of MSP accessory proteins. Subvolume averaging has been applied to individual filaments sampled along their lengths within complex filament meshworks, and the resulting physiological models were compared to earlier models derived using purified MSP, including a helical reconstruction of filaments polymerized in ethanol (King et al. 1992. JCS 101:847) and an x-ray crystal model of MSP subfilaments (Bullock et al. 1998. NSB 5:184). Comparisons suggest important differences between filaments formed under physiological and nonphysiological conditions. There are currently six *Ascaris* sperm proteins known to modulate MSP filament dynamics in sperm; these same proteins are absent from filaments prepared using purified MSP. Comparing MSP filament models with and without these accessory proteins allows us to map the binding sites of these accessory proteins and provides a greater understanding of how they effect MSP filament dynamics and influence motility. Supported by NIH Grant R37 GM29994 and by the American Heart Assoc.

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Control of Directionality of Individual Kinesin-5 Motors

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Kinesin-5 motors fulfill essential roles in mitotic spindle morphogenesis and dynamics as slow, processive microtubule (MT)-plus-end directed motors. The Saccharomyces cerevisiae kinesin-5 Cin8 was found, surprisingly, to switch directionality. Here we have examined Cin8 directionality control using single-molecule fluorescence motility assays and live-cell microscopy. On spindles, Cin8 motors mostly moved slowly towards the midzone, in the plus-end direction of the interpolar MTs. Occasionally, Cin8 also moved faster towards the spindle poles, in the minus-end direction of the MTs. In vitro, individual Cin8 motors could be switched by ionic conditions from rapid and processive minus-end to slow plus-end motion on single MTs. At high ionic strength, Cin8 motors rapidly alternated directionalities between antiparallel microtubules, while driving steady plus-end relative sliding. Deletion of the uniquely large insert in loop 8 of Cin8 induced bias towards minus-end motility and affected the ionic-strength dependent directional switching of Cin8 in vitro. In vivo, the deletion mutant exhibited reduced midzone-directed motility and efficiency to support spindle elongation, indicating the importance of directionality control for the anaphase function of Cin8.

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Myosin-6 Mobility at the Plasma Membrane of Cultured Mammalian Cells

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Myosin-6 is a reverse-directed, actin-based, molecular motor responsible for moving cellular cargo towards the minus-end of actin filaments. It is involved in clathrin-mediated endocytosis and intracellular transport. We have used TIRF microscopy to study the movement and localisation of the ''short insert'' isoform of eGFP-myosin-6 within live 3T3 fibroblasts and human endothelial cells. Individual GFP tagged myosin-6 particles were automatically tracked by computer. Diffusion of membrane associated proteins is usually controlled by lipid mobility and individual trajectories follow a simple ''random walk'' in which mean squared displacement (MSD) is proportional to time interval (dT) and the lateral diffusion coefficient. We found the movement of myosin-6 shows anomalous diffusive behaviour and D_{lat} values are better described by a single exponential function, with a significant fraction of molecules (~30%) having $D_{lat} < 0.02\ \mu m^2\ s^{-1}$. Close examination of individual trajectories revealed that myosin-6 molecules exhibit periods of free movement interspersed with intervals in which motion is arrested. This phenomenon has been described previously as ''transient confinement''. The length of time during which movement of myosin-6 molecules were arrested had an exponential lifetime distribution ($t_{1/2} \approx 250$ ms). The number of observed particles decreased exponentially with time due to photobleaching. However, the shape of the intensity distribution of the individual particles remained constant and was well described by a fit to the sum of two Gaussian terms.

We conclude that myosin-6 molecules associate with the plasma membrane and bind intermittently with immobile sub-cellular structures, which might be either the actin cytoskeleton or trans-membrane anchoring proteins. The ''short insert'' splice variant of myosin VI is predominantly monomeric.

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Using a Non-Averaged Displacement Analysis to Characterize Multiple Populations of Single Molecule Motions

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Microscopic motions of fluorescently labeled single molecules often switch between bound, freely diffusing, and uni-directionally moving states, and in many systems (see below) determining the kinetics of transitions between