to wildtype cells. Moreover, the distinct effect of condensin I and condensin II is also discussed. Our studies provide a quantitative analysis of the effect of condensin on mitotic chromosome condensation.

79-Plat
Organization and Dynamics of the Living E. Coli Nucleoid at High Resolution in Space and Time
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Living fluorescently-labeled E.coli nucleoids were imaged at ~75nm and >2sec resolution. Non-replicating single-chromosome nucleoids are fat helicoidal ellipsoids. They are denser centrally than peripherally and have a strong intrinsic tendency to split longitudinally into two, sometimes more, subellipsoids. Nucleoid density distributions and splitting patterns fluctuate on <5s time scales, dynamically modulating morphology. Analogous helicoids occur throughout the cell cycle. Thus: the E.coli nucleoid is physically coherent object and cannot be modeled as a randomly ordered entropic polymer. Diverse evidences suggest that the nucleoid is stiff, longitudinally and radially, and is trapped within the cell cylinder by radial confinement. We propose that: (i) the nucleoid self-assembles into longitudinal bundles via association of short negatively-supercoiled plectoneme segments; (ii) bundling is opposed by inter-segment repulsion; and (iii) the shape and mechanical properties of bundles underlie the shape and mechanical properties of the helicoid. Nucleoid shapes and bundle patterns were examined throughout the cell cycle. Replication initiates in the helicoid groove towards its old-pole end. Early in replication, straight fingers of newly-synthesized material dynamically protrude and retract, with protrusions curving around the cell periphery. Overall, replication-linked sister segregation is seen to comprise three analogous cycles. In each cycle, a single fat helicoid develop. Then, concomitant with loss of programmed inter-sister snaps, the shape evolves into two thinner spatially-separated helicoids. We propose that genomic contiguity biases longitudinal bundling to give individualized sister shapes (''sister individualization'') and that sisters are driven into an end-to-end relationship, suitable for clean segregation, by the mechanical stress of radial confinement.This scenario explains chromosome dynamics in diverse bacteria. Conversely, bacteria may occur as rods, spirals and spheres because these are the three shapes that promote clean symmetrical sister separation. Implications for eukaryotic chromosomes can be envisioned.

80-Plat
Mechanical Stress on Double Stranded DNA Drives Homology Recognition
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RecA performs a homology search that compares a single stranded DNA (ssDNA) to a double stranded DNA (dsDNA) and accurately recognizes homologous sequences in a background containing non-homologous and near-homologous sequences. This recognition process can occur in the absence of ATP hydrolysis, indicating that it must be thermodynamically reversible and free energetically favorable. In this poster, we examine the mechanism by which RecA performs comparisons over sequences that are much longer than an individual protein while avoiding kinetic trapping in regions with local accidental homology. We present a model of RecA bound to DNA that uses a simplified version of its known structure to calculate the distribution of mechanical stress in the protein/DNA complex. In addition, we use computer simulations to model the dynamic polymerization process. We propose that the lattice mismatch between the more strongly and weakly bound strands of DNA results in a mechanical stress that increases non-linearly with the number of bound base pairs. The distribution of stress combined with an iterative homology search may explain several previously unexplained features of the structure and function of RecA. The model is consistent with mechanical data obtained from magnetic tweezer measurements of polymerization of RecA on double stranded DNA (dsDNA) and the extension of RecA-ssDNA filaments on dsDNA. RecA's mechanism can also be instructive for artificial self-assembled systems.

Platform: Muscle: Fiber & Molecular Mechanics & Structure

81-Plat
Subnanometer Structure of the Actin/Myosin/Tropomyosin Complex
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Actins and myosins play a central role in life as they form the molecular motors that transform chemical energy into movement. Without them, we would be confined to a very static existence.
To fully understand how the interplay between actin and myosin leads to the conversion of the energy stored in ATP into mechanical energy, one needs structural information. Therefore, we have set out to determine the complex structure of the three major muscle proteins (actin, myosin and tropomyosin) using cryo electron microscopy and iterative helical real-space reconstruction. Using state of the art instruments and reconstruction algorithms we have determined a sub-nanometer resolution electron density map of the complex. With the help of electron-density guided flexible fitting we have subsequently refined the structure to obtain a quasi-atomic resolution structure of the full complex which enabled us, for the first time, to visualize interactions between actin and myosin that have been postulated by computational methods beforehand. In addition, we could also identify a novel interaction between myosin and tropomyosin.

82-Plat
Structural Model of the Pre-Powerstroke State of the Actomyosin Complex
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We created a structural model of the ADP.Pi pre-powerstroke state of Dictyostelium myosin motor domain complexed with actin trimer by an in silico protein docking procedure followed by a long timescale molecular dynamics relaxation. Furthermore, we also modeled the ADP.Pi down lever state of myosin motor domain and the rigor complexes using 1q5q (apo structure of Dictyostelium myosin motor domain) or squid myosin S1 (3I5G) atomic structures and actin trimer model. During the molecular dynamics of the apo myosin (1q5q) complexed with actin trimer the actin binding cleft of myosin motor domain spontaneously closed and the relaxed actomyosin rigor structure fits well with the structural rigor model determined by EM. The analysis of the ADP.Pi pre-powerstroke actomyosin complex showed that actin spontaneously induces significant conformational changes in the myosin motor domain. Most strikingly, actin further closes the closed switch 2 loop coupled with a further up movement of the lever. Interestingly, if the interaction between the N-terminal region of actin and myosin activation loop is interrupted by a single mutation (K520N) this conformational change does not occur upon actin binding. Furthermore, actin binding rearranges correlating movements of different myosin motor regions which effect was significantly reduced in the mutant. Recently we shoved experimentally that the interaction between actin and the activation loop is responsible for channeling the enzymatic pathway of actomyosin into the effective powerstroke path. The further closure of switch 2 induced by actin indicates that cocking is induced by actin mainly through the actin binding of activation loop. In order to test the indicated conformational change we have produced a myosin motor domain containg a FLASH and REASH probes located at the N and C-terminus, respectively, to sensitively follow the lever movement upon actin binding by FRET.

83-Plat
The Two-Dimensional Kinetics of Binding and Unbinding are Both Regulated by Myosin's Actin-Binding Loop
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Binding of myosin to actin is a multi-step, ionic strength-sensitive process for which the kinetic properties of bond formation and rupture have been studied in solution. However, the solution (three dimensional) environment is a poor model of the pseudo-two dimensional geometry in which myofilament proteins interact *in vivo*. We therefore investigated the rate of actomyosin bond formation and rupture in a two-dimensional setting using dynamic force spectroscopy at physiological and reduced ionic strength. We previously showed that catch bond rupture is slowed at physiologic ionic strength (145 mM KCl) when compared to low ionic strength (25 mM), and that bond lifetime is maximal at the isometric force generated by a single myosin molecule. In the current study we confirmed this result and sought to determine whether this difference is mirrored in the rate of actomyosin bond formation. A laser trap was used to measure the time to bond formation (t_b) between actin filaments and nucleotide-free heavy meromyosin (HMM) over a range of compressive loads. Two dimensional on-rates were determined from t_b and were increasingly force-dependent with decreasing ionic strength. We examined the effect of ionic strength on the initial long-range ionic interaction constituting the first step of actomyosin binding by targeted tryptic digestion of HMM's actin-binding loop. Our data at physiologic ionic strength suggest that the actin-binding loop contributes to the decreased 2D rate and load-dependence of bond formation. This is matched by reduced rates of actomyosin unbinding. These data