View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

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
Jay Fisher, Aude Bourniquel, Guillaume Witz, Mara Prentiss,
Nancy E. Kleckner.
Harvard University, Cambridge, MA, USA.
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
Efraim Feinstein, Claudia Danilowicz, Mara Prentiss.
Harvard University, Cambridge, MA, USA.
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
Elmar Behrmann[1], Krishna Chinthalapudi[2], Sarah M. Heissler[2],
Pawel A. Penczek[3], Hans G. Mannherz[4], Dietmar J. Manstein[2],
Stefan Raunser[1].
[1]MPI of Molecular Physiology, Dortmund, Germany, [2]Hannover Medical School, Hannover, Germany, [3]Houston Medical School, Houston, TX, USA, [4]Ruhr University, Bochum, Germany.
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
Yang Zhenhui, Boglarka Varkuti, Anna Rauscher, Miklos Kepiro,
Andras Malnasi-Csizmadia.
Eotvos University, Budapest, Hungary.
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
Amy M. Clobes, William H. Guilford.
University of Virginia, Charlottesville, VA, USA.
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

provide useful parameters for modeling studies and suggest that the actin-binding loop of myosin contributes to both the binding and unbinding of myosin from actin.

84-Plat
Tropomyosin Movement on F-actin Analyzed by Energy Landscape Determination

Marek Orzechowski[1], Stefan Raunser[2], Stefan Fischer[3], William Lehman[1].
[1]Boston University School Medicine, Boston, MA, USA,
[2]Max Planck Institute of Molecular Physiology, Dortmund, Germany,
[3]Heidelberg University, Heidelberg, Germany.

Muscle contraction is regulated by movement of tropomyosin over the surface of actin filaments. At low-Ca^{2+}, tropomyosin blocks myosin-binding on actin, whereas, in a two-step process, Ca^{2+}-saturated troponin and myosin open the myosin-binding site leading to contraction. No obvious steric obstructions or geometrical barriers on actin limit such tropomyosin movement. However, lacking atomic models for these transitions, the pathways taken by tropomyosin during regulatory movements are uncertain. Here, end-points for regulatory transitions were determined by fitting tropomyosin to EM reconstructions. Reconstructions of negatively-stained low-Ca^{2+} thin filaments yield an atomic model very close to that described for troponin-free actin-tropomyosin by Li et al. (2011), while comparable high-Ca^{2+} filament maps suggest an azimuthal sliding of tropomyosin parallel to its superhelical path. Additionally, Raunser et al. (2012-Biophys. Soc. abst.) describe an atomic model of tropomyosin based on high-resolution cryo-EM reconstructions of myosin-decorated filaments, showing expected azimuthal movement and pronounced ~15Å axial displacement of tropomyosin toward the pointed-end of the thin filament. To evaluate transitions between these various regulatory positions, we explored the energy landscape between ''end-states'' over a comprehensive grid of 832 tropomyosin locations relative to F-actin coordinates. The position of tropomyosin was varied azimuthally and axially relative to F-actin, and then the structure energy-minimized. The resulting electrostatic energy landscape shows a wide energy basin with a minimum centered near the blocked-state. The width of this basin indicates that large azimuthal and axial oscillations of tropomyosin are possible. By contrast, in the myosin-induced open-position, tropomyosin is located at an energy peak, representing a region with no obvious complementary electrostatic interactions between tropomyosin and F-actin. Our results therefore suggest that the open-position is reached only because of tropomyosin interaction with myosin, while binding of tropomyosin to the F-actin surface becomes less important.

85-Plat
Structural Changes in Both the Troponin Complex and the Thick Filament May Underlie Myofilament Length Dependent Activation

HsiaoMan Hsu[1], Younss Ait Mou[2], Thomas C. Irving[1], Pieter P. de Tombe[3].
[1]Illinois Institute of Technology, Chicago, IL, USA, [2]Loyolla University Chicago Stritch School of Medicine, Maywood, IL, USA, [3]Loyolla Unversity Chicago Stritch School of Medicine, Maywood, IL, USA.

The main cellular mechanism that underlies the so-called ''Frank-Starling Law of the Heart'' is an increase in the responsiveness of cardiac myofilaments to activating Ca^{2+} at longer sarcomere lengths (SL). The structural basis of this ''Length Dependent Activation'' (LDA) is not known. 2D X-ray diffraction patterns were obtained using the BioCAT beamline 18ID at the Advanced Photon Source from electrically stimulated (0.2 Hz) intact, twitching papillary muscle isolated from rat hearts during a 10 ms time window in diastole just prior to electrical stimulation. Diffraction patterns were compared from muscles that were stretched to L_{max} (SL= ~2.3 μm) to those taken following a quick release to slack length (SL=~1.9 μm). We previously reported that myosin heads moved radially inwards at longer SL suggesting that an increased radial extent of crossbridges at longer length cannot explain increased calcium sensitivity so other explanations must be sought. It is known that changes in isoform composition of the troponin complex can markedly affect calcium sensitivity but the role of troponin in the length sensing mechanism underlying LDA is not clear. Here we analyzed the meridional patterns which showed that the 3rd order troponin repeat distance, the 3rd - order troponin reflection intensity and the 2nd order myosin (''forbidden'') meridional reflection all increased significantly ($P < 0.01$) at L_{max} as compared to slack length. Thus, stretching intact heart muscle in diastole induces changes in the structure of both the thick filaments and the thin filaments. It appears, then, that the length sensing mechanism underlying LDA must involve connections of some kind that transmit strain between the thick and thin filament that alter the structure of the troponin complex and, presumably, myofilament contractile properties. Supported by **NIH HL075494** and RR08630.

86-Plat
Myosin Heavy Chain Isoforms Influence the Magnitude of Stretch Activation in Drosophila Muscles

Cuiping Zhao, Douglas Swank.
Department of Biology, Rensselaer Polytechnic Institute, Troy, NY, USA.

The stretch activation (SA) mechanism, a general property of all muscle types, is most prominent in muscles that generate power through cyclical contractions, such as insect indirect flight muscles (IFM) and vertebrate cardiac muscles. SA is defined as phase 3, a delayed tension increase, of the tension transient following a muscle stretch. We are testing our hypothesis that myosin heavy chain isoforms help set SA magnitude and hence power generating ability in different muscle types. The *Drosophila* thorax contains two muscle types, IFMs and jump muscles. In IFMs, the magnitude of SA is great enough to enable net positive power generation when it is fully calcium activated. In contrast, jump muscles display very little stretch activation and cannot generate net positive power when fully activated. We found that when an embryonic myosin isoform (EMB) is transgenically expressed in the jump muscle, the muscle is transformed and behaves like a moderately stretch-activatable muscle. The transformed muscle can now generate positive power and SA magnitude is increased by ~60% at 0mM Pi and ~350% at 16mM Pi. This shows that SA magnitude is influenced by myosin isoforms. We found that power generation is [Pi] dependent as $\geq$ 2mM Pi is required for power production in jump muscles expressing the EMB isoform. Expressing the IFM isoform in jump muscles did not enable positive power generation or increase SA magnitude. To further test our hypothesis, we are expressing the jump myosin isoform in IFMs to determine if this reduces IFM SA magnitude. We conclude that myosin isoforms can influence the magnitude of SA, but are not the only mechanism responsible for natural differences in SA magnitude.

87-Plat
Half-Sarcomere Mechanics and Energetics Indicate that Myosin Motors Slip Between Two Consecutive Actin Monomers during their Working Stroke

Marco Caramani, Luca Melli, Mario Dolfi, Vincenzo Lombardi,
Marco Linari.
University of Florence, Florence, Italy.

The coupling between chemical and mechanical steps of actomyosin ATPase cycle was studied in situ by using fast mechanical protocols in Ca2+-activated demembranated fibres from rabbit psoas under sarcomere length control (sarcomere length 2.4 μm, temperature 12°C). We determined the effects of the concentration of inorganic phosphate (Pi) on the force-velocity relation (T-V), on the stiffness-velocity relation (e-V) and on the isotonic velocity transient following a stepwise drop in force from the isometric plateau force (T0) (Piazzesi et al. J Physiol 545:145, 2002). With respect to control (no added Pi), the increase of [Pi] to 10 mM, i) reduced T0 by 50-60%, decreased the curvature of the T-V relation by 30% and increased the unloaded shortening velocity (V0) by 19%; ii) decreased the relative half-sarcomere stiffness at each shortening velocity by an extent that indicates that Pi has little effect on the force per attached myosin motor; iii) did not change the rate of early rapid shortening (phase 2) following the stepwise drop in force, while reduced its size and made the subsequent pause of shortening (phase 3) briefer. Steady state and transient mechanical responses and the known related energetics (Potma and Stienen J Physiol 496:1, 1996) are simulated with a kinetic-mechanical model of the actomyosin ATPase cycle that incorporates Huxley and Simmons mechanism of force generation. Muscle power and efficiency during isotonic shortening at high and intermediate loads can be predicted only if myosin motors at an intermediate stage of both the working stroke and product release can slip to the next Z-ward actin monomer. Supported by MIUR, Ministero della Salute and Ente Cassa di Risparmio di Firenze (Italy).

88-Plat
Myosin-Induced Detachment Causes Differences Between Ensemble and Single Molecule Myosin Kinetics

Sam Walcott[1], Edward P. Debold[2].
[1]UC Davis, Davis, CA, USA, [2]UMass Amherst, Amherst, MA, USA.

Single molecule measurements of mechanochemistry have greatly increased our understanding of muscle contraction. However, since trillions of myosin molecules work together in muscle, extrapolation to in vivo function requires additional understanding of how motors behave in an ensemble. Early findings suggested that myosin behaves similarly at both the single molecule and ensemble levels; but more recent experiments suggest otherwise. Using a combination of simulation and theory, we show that the force-dependence of ADP release