675-Pos Board B475
The Force Production by the Depolymerization Activity of MCAK
Yusuke Oguchi, Seiichi Uchimura, Sergey V. Mikhailenko, Takashi Ohki, Shin'ichi Ishiwata.
During cell division the replicating chromosomes must be precisely arranged and separated polewards. Though many cellular processes involving motility require force generation by motor proteins, the chromosome movement is suggested to use the energy stored at plus ends of the microtubules to which kinetochores are attached. This energy is converted into the chromosome movement via passive couplers, whereas the role of the microtubule-based motors is supposed to be limited to the regulation of microtubule dynamics. The microtubule-depolymerizing kinesins, such as mitotic centromere-associated kinesin (MCAK), which is a founding member of kinesin-13 family, facilitate microtubule dynamics in a spindle and are required for the chromosome congression and segregation; however, the key question - whether the depolymerizing activity generates tension to pull the chromosomes - has remained unsolved. To probe the link between the generation of tension and the microtubule-disassembling activity of MCAK, we developed a single-molecule assay to examine the interaction between a single microtubule and multiple MCAK molecules under the fluorescence microscope equipped with the dual-trap optical tweezers. Here we show that the microtubule-disassembling activity of MCAK generates significant tension. The depolymerization force increases with the number of interacting MCAK molecules. These results provide a simple and attractive model for the generation of the driving force and the regulation of chromosome segregation in a spindle by the activity of MCAK.

676-Pos Board B476
Investigating the Interaction between Kinesin-13s and Microtubules by Single Molecule Fluorescence Polarization Microscopy
Chandrima Chatterjee, Ana B. Asenjo, Vania M. De Paoli, Hernando J. Sosa.
Kinesins are motor proteins that translocate along microtubules (MTs) and assist in transportation of intracellular cargo by utilizing the energy generated from ATP hydrolysis. Interestingly, Kinesin-13s do not walk but depolymerize microtubules at their ends, an activity that is essential for regulating MT dynamics during cell-division. Prior research efforts have shown that Kinesin-13s target MT ends very quickly by the process of one-dimensional diffusion along the MT lattice. In an effort to elucidate the mechanism of such diffusive interactions and MT depolymerization activity, we have investigated the orientation and dynamics of BSR-labeled KLP10A (Drosophila m. Kinesin-13) constructs interacting with MTs in the presence of ATP analogues using fluorescence polarization microscopy (FPM). Unlike conventional kinesins, which show high mobility in nucleotide conditions that induce weak MT binding (presence of ADP), KLP10A shows relatively high fluorescence anisotropy in all nucleotide states investigated, including ADP. Such observations indicate that the nucleotide present has minimal effects on the binding configuration of the majority of KLP10A molecules bound to the MT lattice. However, FPM data acquired at the single-molecule levels suggest that the Kinesin-13 motor domain becomes very mobile (tumbling) when undergoing one-dimensional diffusion. Ongoing experiments with KLP10A constructs of different lengths and mutants will help in identifying the residues in the Kinesin-13 molecule that mediate one-dimensional lattice diffusion.

677-Pos Board B477
In Vivo Role of a Novel Kinesin-13 Microtubule Binding Site
Ana B. Asenjo, Uttama Rath, Dongyan Tan, David J. Sharp, Hernando Sosa.
Kinesin 13 family proteins regulate microtubule dynamics by using the energy of ATP hydrolysis to induce microtubule (MT) depolymerization. They have important roles in regulating MT dynamics during mitosis. Previous work in our lab identified a novel site of interaction of kinesin13 with the MT that leads to the formation of rings wrapping the MT in the presence of AMP-PNP. Several positively charged residues at this site were shown to be required for the formation of the rings but not for depolymerization activity. Here we investigate the effect of replacing these residues on the *in-vivo* function of one of the three Kinesin13s present in *Drosophila* m., KLP10A. We made DNA construct to express GFP-labeled wild type KLP10A and a mutant in which three crucial residues at the novel binding site were replaced by alanines. . We expressed these constructs in S2 cells in the presence or absence of endogenous KLP10A. We found that cells over-expressing the mutant protein were two times more likely to have mitosis abnormalities than cells over-expressing the wild type protein. In addition, in cells where the endogenous protein had been depleted by siRNA, ectopic expression of the mutant protein was less effective rescuing mitotic defects caused by the depletion than ectopic expression of wild type KLP10A. These results indicate that kinesin-13-MT interactions mediated by the novel binding site are important for normal cellular progression through mitosis.

Muscle: Fiber and Molecular Mechanics & Structure I

678-Pos Board B478
Fluctuations in Muscle
Michael L. Shurr.
1. Myofibril is an absolutely paradoxical design of the Nature. A human being neither has created nor will construct such a structure (sequentially connected engines) at all. Actually, each sarcomere is an individual self-contracting force element. Since the force along the myofibril is apparently constant, each sarcomere produces force; However, acceleration of the left end of each subsequent sarcomere is higher than that of previous one, because though it contracts by itself (in a fixed, relative to it, coordinate system), its mass center moves as it is pulled by all the previous sarcomeres. Hence, it follows that the acceleration and the force along the myofibril could not be constant!? Yet, the myofibril is a reality and our muscles are quite capable of working.
2. In the myofibril performance a decisive role is played by fluctuations of the number of attached cross-bridges. As a result, one, the most powerful sarcomere turns out to be a driver.
3. The sarcomere proper is a thermodynamic system. It is the point of view that must the base for its treatment.
We would like to emphasize that sarcomere is analogous to rubber and cite Sr. R Feinman: «Rubber consists of long organic molecules connected with each other by crosspieces (crossbridges). Thermal vibrations of these molecules make them tend to roll up, which is just the reason for the appearance of contracting elastic force of rubber.».
We consider accounting for the vibrations of the actin-myosin system, which interacts with the sarcoplasmic solution, to be of principal importance.
Thus, part of energy of the actin-myosin system is oscillatory. One can show that energy of the ensemble of oscillators interacting with thermostat (sarcoplasmic solution) significantly increases owing to fluctuations.

679-Pos Board B479
Quantification of Cross-Bridge Cycling between the Different Physicochemical Conformations by Optical Means
Tamar Harary, **Amir Landesberg**.
The transmitted light polarization is determined by sarcomere alignment, sarcomere length (SL) and cross-bridge (XB) distribution between the various physicochemical conformations. **Aims:** To quantify the changes in cardiac muscle optical properties during contraction and to differentiate between the effects of changes in SL and those of XB cycling. **Methods:** Thin trabeculae (n=6) were isolated from rat right ventricles. SL was measured by laser diffraction technique and controlled by a fast servomotor. The direction of the incident polarized HeNe laser light was set to 45^0 relative to the fiber axis. The changes in the transmitted light polarization were measured during rest, twitch contraction, sarcomere control isometric contractions (1.97μm), and at different calcium concentrations (0.75, 1.5, 4.5 [mM]). **Results:** The time to peak change in the transmitted light intensity preceded (50 ± 10msec) the peak force (120msec), and the transition back to baseline intensity lagged behind force relaxation. There was a tight correlation between the rate of changes in the light intensity and the rate of force development. The degree of polarization decreased during force development, reaching a minimal value close to the peak force. These observations could not be attributed to changes in the SL since similar phenomena were observed during isometric sarcomere contractions, and the variations in the degree of polarization were even greater (13%) during sarcomere isomeric contractions. The role of the XBs was furtherer validated by imposing isometric contractions at the same SL but different force levels with different extra-cellular calcium concentrations. **Significance:** optical measurements provide additional information about XB dynamics that differ from the observed dynamics of force or stiffness measurements, and it can be used for quantifying cardiac muscle activation and XB cycling.

680-Pos Board B480
Active Stretch does not Affect the Rate of Force Redevelopment in Rabbit Skinned Fibres
Venus Joumaa, Walter Herzog.
University of Calgary, Calgary, AB, Canada.
The steady state force produced by a muscle following active stretch is greater than the corresponding purely isometric force. This phenomenon is called "Residual Force Enhancement" (RFE). Most of the proposed mechanisms of RFE were either related to the development of structural non-uniformities, or