the thin filament, whereas the dilated cardiomyopathy mutations (TnTR141W and TnTdeltaK210) accelerated the rate of Ca2+ dissociation from the thin filament. The Ca2+ sensitizing compound bepridil had no effect on the rate of Ca2+ dissociation in either thin filaments or myofibrils, but engineered mutations of TnC can accelerate or slow the rate. Finally, the alpha and beta isoforms of tropomyosin slowed and accelerated the rate of Ca2+ dissociation from the thin filament, respectively. Thus, multiple factors can modulate the rate of Ca2+ dissociation from the thin filament.

2593-Pos Board B563

Kinetics of Ca^{2+} Dissociation-Induced Structural Transitions of Cardiac Thin Filament

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Ca^{2+} induces structural transitions within the cardiac thin filament. The structural kinetics at the troponin-actin interface was investigated by Förster Resonance Energy Transfer (FRET) to understand the molecular basis underlying thin filament regulation. The kinetics of the Ca^{2+}-induced conformational changes at the cTnC N-domain, the cTnC-cTnI and the cTnI-actin interfaces were studied. The structural transition of the cTnC N-domain was examined by monitoring FRET between a donor (AEDANS) attached to one cysteine and an acceptor (DDPM) attached the other cysteine mutant of cTnC(13C/51C). The cTnC-cTnI interactions were investigated by monitoring the distance changes from cTnC(89C) to cTnI(151C) and cTnI(167C). Both cTnI(151C) and cTnC(167C) were labeled with AEDANS as FRET donor and cTnC(89C) was labeled with DDPM as the FRET acceptor. These two labeled cTnI mutants were also used to monitor Ca^{2+}-induced distance changes from cTnI residues 151 and 167 to the cysteine residue 374 of actin labeled with DABM as the FRET acceptor. Results from FRET Ca^{2+} titrations and stopped-flow kinetic measurements demonstrated that different structural transitions have different Ca^{2+} sensitivities and different Ca^{2+} dissociation-induced kinetics. Structural transitions involving the regulatory region and the mobile domain of cTnI occurred at fast kinetic rates, while the structural transitions involving transversal movement of the cTnI inhibitory region occurred at slow kinetic rates. Our results suggest a two-step deactivation of the thin filament upon Ca^{2+} dissociation. The first step may involve rapid binding of the mobile domain of cTnI to actin, which was kinetically coupled with the conformational change of cTnC N-domain and dissociation of the regulatory region of cTnI from the cTnC hydrophobic pocket. The second step involved the inhibitory region of cTnI switching its interacting from cTnC to actin. The latter processes may participate in regulating crossbridge kinetics.

2594-Pos Board B564

An Internal Domain of Beta Tropomyosin Increases Myofilament Calcium Sensitivity

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Tropomyosin (TM) is involved in calcium mediated muscle contraction and relaxation in the heart. Striated muscle alpha TM is the major isoform expressed in the heart. Expression of striated muscle beta TM in murine myocardium results in a decreased rate of relaxation and increased myofilament calcium sensitivity. Replacing the carboxyl terminus (amino acids 258-284) of alpha TM for beta TM (a troponin-T [TnT] binding region) results in decreased rates of contraction and relaxation in the heart and decreased myofilament calcium sensitivity. We hypothesized that the putative internal TnT binding domain (amino acids 175-190) of beta TM may be responsible for the increased myofilament calcium sensitivity observed when the entire beta TM is expressed in the heart. To test this hypothesis, we generated transgenic mice that express a chimeric TM containing beta TM amino acids 175-190 in the backbone of alpha TM (amino acids 1-174 and 191-284). These mice express 16% - 57% chimeric TM, and they do not develop cardiac hypertrophy or any other morphological changes. Physiological analysis shows these hearts exhibit systolic and diastolic dysfunction and a positive response to isoproterenol. Skinned fiber bundle analyses show a significant increase in myofilament calcium sensitivity. Biophysical studies demonstrate that the exchanged amino acids do not influence the flexibility of TM. This is the first study to demonstrate that a specific domain within TM can increase calcium sensitivity of the thin filament. Further, these results enhance the understanding of why TM mutations associated with familial hypertrophic cardiomyopathy also demonstrate increased myofilament sensitivity to calcium.

2595-Pos Board B565

The Assessment of Uncertainty in Measurement of Cholesterol: A Model of Calculation

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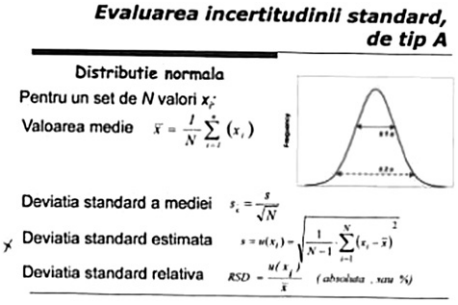
The objective of this work was to identify all the components of uncertainty in measurement of Cholesterol, undergoing a reasonable estimation of results in the acceptable method.
Material and Method:
For identification of the uncertainty in measurement of Cholesterol in laboratory, running Hitachi 912 Roche Analyzer, was necessary the Standard Cholesterol (ST).
Results:
Was calculated, in function of Standard Deviation (SD) and Student Factor (t), by estimation (SD * t), Compound Uncertainty of ST, assembling Uncertainty of A type and Uncertainty of B type, in value of 11 mg%.
Conclusion:
Budget of Uncertainty, in assessment of Cholesterol was established to a permitted error of 11% in normal range and under cut-off.



Microtubules & Microtubule-associated Proteins

2596-Pos Board B566

Tau Directly Inhibits The On-rate Of Kinesin, For Microtubules, During Transport

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Kinesin belongs to a class of microtubule (MT) based molecular motors, which can facilitate the intracellular transport of vesicle bound cargo and organelles throughout the cell. This vital function is especially pronounced in the neuron, where transport down exceedingly long processes, such as axons and dendrites, must be efficiently accomplished and cannot be explained be diffusion alone. Like most cellular processes, regulation is a fundamental aspect of kinesin mediated intracellular trafficking and to date many modes to regulate kinesin have been elicited. Recently the microtubule associated protein (MAP), tau, has been implicated in playing a central role in the regulation of kinesin mediated transport in the neuron. Tau has previously been shown to reduce the procesivity and attachment frequency of kinesin motors on MTs. Although it has been demonstrated that tau has a dramatic affect on kinesin based transport, the mechanism by which this occurs is presently unknown. Using stopped-flow rapid kinetics, we demonstrate that tau directly affects the on-rate of kinesin for MTs causing the motor to dissociate from the MT track. Because kinesin releases ADP at a greatly accelerated rate in the presence of MTs, we can effectively monitor the on-rate of kinesin for MTs by following the release of the fluorescent ADP analog mantADP. We demonstrate the on-rate of kinesin, for MTs, is reduced by tau in both a concentration and isoform specific manner.

2597-Pos Board B567

Synchrotron X-ray Scattering Study of the Effects of Microtubule-associated-protein (MAP) Tau on Interprotofilament and Intermicrotubule Interactions

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Neuronal microtubules (MTs) are 25 nm protein nanotubes used as tracks for intracellular trafficking of biomolecules, for example, those involved in transmitting signals between neurons. Distinct members of MAP tau isoforms regulate microtubule assembly and stabilization. Altered tau-MT interactions lead to MT depolymerization and tau tangles, which are implicated in a large number of neurodegenerative diseases. We describe our recent findings about the effect of human wild type MAP tau on interprotofilament and intermicrotubule interactions, by using synchrotron small angle x-ray scattering. Supported by DOE DE-FG02-06ER46314, NSF DMR-0503347, NIH GM-59288, NIHI RO1-NS35010 and NS13560.

2598-Pos Board B568

Comparison of Microtubule Dynamics for A- and B-Lattice Geometries

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Accurate quantitative interpretation of experimental data and prediction of the effects of microtubule-targeted anti-mitotic drugs require a detailed model of the events that occur at microtubule ends. Before searching the large parameter space of a model with few constraints on lattice symmetry, binding site configuration, GTP-hydrolysis rate, and oligomerisation state of the associating and dissociating species, we performed an extensive, systematic investigation into the dynamics of a series of simplified models with significantly smaller parameter spaces. The models had regular A or B-lattice geometries, tightly coupled GTP-hydrolysis, and association-dissociation events involving the formation or breakage of just two lateral bonds. GTP-hydrolysis weakened the two lateral bonds to the β-tubulin subunit by 4.6 k_BT, in either a balanced (+2.3 k_BT each) or an unbalanced way (+4.6 k_BT for one and 0 for the other bond). Association rate constants were 1 $\mu M^{-1}s^{-1}$, and dissociation rates were thus dependent on the lateral bond energies. We observed the following:

1. Values for C_C (the concentration of free tubulin-GTP at which the net growth is zero) varied from 1.2 to 80 μM
2. All configurations showed discernable phases of growth (G) and shrinkage (S) around their C_C
3. Effective growth rates at C_C (average growth rate during the G-phase divided by the maximum attainable growth rate at that C_C) varied from less than 0.1 in most of the B-lattice geometries to 0.9 in the A-lattices with a balanced effect of hydrolysis
4. G-phase lifetimes were relatively short (10-15 s), and growth was significantly more uniform in the balanced A-lattice geometries, compared with those in the unbalanced geometries (lifetimes > 100 s)

Thus, balanced A-lattice configurations support efficient growth on relatively unstable microtubule ends, whereas most other configurations grow less efficiently on more stable ends.

2599-Pos Board B569

Response of the Mitotic Spindle to Mechanical Force

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Cell division is an inherently mechanical process, from chromosome congression, error correction and segregation to furrow ingression. Although the molecules involved in cell division are becoming better characterized, surprisingly little is known about the underlying mechanical principles and interactions. The spindle is a complex assembly and its response to mechanical force should yield insight into its structure, the mechanisms governing its shape and size, and how forces are transmitted from the spindle apparatus to the chromosomes. Here, we develop an assay to mechanically flatten mitotic spindles in live mammalian cells and use fluorescence microscopy to monitor the response of the microtubule cytoskeleton and kinetochores. We show that, upon flattening, the spindle deforms asymmetrically: it widens rapidly as the kinetochore-microtubule bundles pivot around the poles, and lengthens slowly in a tubulin polymerization-dependent manner. Interestingly, spindle length can double reversibly under the mechanical perturbation, providing insight into spindle size determination. In addition, we find that kinetochore motion is robust to changes in spindle shape and size, and to forces resulting in drastic bends of kinetochore-microtubule bundles, suggesting that kinetochore motion is locally driven. Finally, the data point towards a framework where mechanical forces are locally transmitted and generated by the spindle and the method introduced provides a useful tool to probe mechanical interactions between spindle components.

2600-Pos Board B570

Challenges In Modeling Chromosome-driven Mitotic Spindle Formation

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A mitotic spindle is a regular structure within a cell, consisting of oriented microtubule fibers. It plays a fundamental role in chromosome separation during cell division. Forming a spindle pattern is a major structural step towards mitosis. We have developed a biophysical non-equilibrium thermodynamic model to describe in vitro chromosome driven spindle formation experiments in Xenopus extracts. Our modeling work, as well as the work of others such as Nédélec and collaborators, has shed considerable light on this process. Our modeling analysis has produced results that agree in several respects with experimental findings. We believe, however, that there are a number of challenges that must be addressed for spindle modeling to continue to be a useful tool for understanding this fundamental biological process. A biophysical model for spindle formation requires detailed biological hypotheses determining the behavior of key model elements. Current modeling work has shown some deficiencies in our understanding of particular problems. In particular, better biological hypotheses are needed to describe how molecular motors behave near the endpoints of microtubules and how those motors influence microtubule dynamic instability. We will detail what we believe are important problems needing better biological hypotheses. Accurate numerical modeling based on biophysical models of mitosis is challenging because the models must simultaneously represent thermal diffusion effects that happen in microseconds as well as spindle formation processes that take minutes or even hours. We will discuss our work on numerical algorithmic improvements that will greatly speed simulations without sacrificing biophysical model properties or numerical accuracy.

2601-Pos Board B571

Antimitotic agent alters MIP levels In breast cancer cells

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Progression through mitosis requires a balance of active microtubule-interacting proteins (MIPs) that stabilize and destabilize microtubules in mitotic spindles. Molecular interactions between MIPs and tubulin or microtubules are important for cell cycle progression. Insights into these interactions can contribute to understanding the mechanisms underlying cell cycle interference by antimitotic agents that halt cell cycle progression in mitosis. We examined the direct interaction of the antimitotic agent, vinblastine with tubulin and stathmin using AUC, in order to understand how changes in stathmin levels during the cell cycle might affect the cellular drug response. Vinblastine acts during G2/M phase of the cell cycle and reduces microtubule dynamics. At high doses it destabilizes microtubules in mitotic spindles. We found in vitro that stathmin reduces the potency of vinblastine. Vinblastine was found to compete for tubulin-stathmin oligomers, at the same time as it induced tubulin spiral formation. To extend these data to a cellular context, we investigated changes in intracelluar MIP levels in response to paclitaxel, an antimitotic agent known to stabilize microtubules at high concentrations. Using qRT-PCR we found that paclitaxel treatment of human breast cancer MCF7 cells leads to a significant reduction in MAP4 and stathmin mRNA levels. Interestingly, the levels return to pre-paclitaxel treatment levels after a 4-day drug washout, suggesting that paclitaxel alters transcript levels. We found that the ratios of MAP4/stathmin increased after or during drug treatment. These data suggest that changes in MIPs levels alter the cellular response to drugs. These results also suggest that disruption of the cell cycle by antimitotic agents can alter the relative amounts of MIPs and thus affect the balance needed for normal progression through the cell cycle. These results must be taken into account when modeling the cellular response to antimitotic drugs.

2602-Pos Board B572

Theoretical Description of Microtubule Dynamics in Fission Yeast During Interphase

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Fission yeast (S. pombe) is a unicellular organism with a characteristic cylindrical shape. Cell growth during interphase is strongly influenced by microtubule self-organization - a process that has been experimentally well characterised. The microtubules are organized in 3 to 4 bundles, called "interphase microtubule assemblies" (IMAs). Each IMA is composed of several