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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