switching system into the microtubules enables high efficient regulation of kinesin motor activity.

In this study, we have done the experiments on incorporation of photochromic molecule into wild type microtubules and gliding assay of them under UV and VIS light irradiation. The gliding speed of the wild type microtubules modified with PAM or 3,3-dimethyl-6'-nitro-1-[2-[3-(3-pyryline)-2,5-dion] -1-v] propanoloxyl]ethylindoline -2-spiro-2(2H)-chromone (MASP) did not change. The results suggested that modification of the intrinsic reactive cysteine residues on the surface of microtubules does not affect motor activity of kinesin. Therefore, currently we are trying to prepare mutant microtubules that have an additional single reactive cysteine residue at the region near the kinesin binding site.

2439-Pos Board B425
Preparation and Polymerization of Tubulin of Ginkgo Biloba and its Interaction with Rice Plant Kinesin
Seigo Iwata, Nozomi Umezu, Kazunori Kondo, Shinsaku Maruta.
Previously, we have expressed novel rice plant specific kinesins and studied their biochemical characterization. The plant kinesins showed very unique properties. Especially ATPase activities of the kinesins were relatively much lower than that of conventional kinesin. Moreover, the kinesins did not show motility activity on the microtubules prepared from porcine brain. Although the structure of tubulin is well conserved, it is demonstrated that the plant tubulin has different characteristics from tubulin derived from animal. Therefore, plant kinesin may be more compatible with plant tubulin than animal tubulin. In this study, we prepared the plant tubulin from the pollen of *Pear* and *Ginkgo biloba*. The tubulin was extracted from the acetone powder of pollen. Subsequently, the tubulin was purified with DEAE Sephadex A-50 chromatography, Sephacryl S-300 gel filtration, and Mono Q anion exchange chromatography. And we also prepared the plant tubulin from the leaf of *Ginkgo biloba* utilizing the TCA/Phenol method. Polymerization of the purified plant tubulin to microtubule was monitored by measuring the increase of absorption at 350 nm. Negative staining electron microscopy analysis revealed the microtubule configuration. In preliminary experiments, the ATPase activity of rice plant kinesin K16 was activated by plant tubulin more significantly than that of animal tubulin. These results suggested that plant kinesin we have prepared is more compatible to plant kinesins than kinesin derived from animal.

2440-Pos Board B426
Katin P60 and Fidgetin, Variations on Microtubule-Severing Mechanisms
Juan D. Diaz.
The dynamic properties of microtubule polymers are tightly regulated by the cell, often through the use of microtubule-associated proteins (MAPs). A novel class of microtubule-associated protein causes the severing of microtubules and are called microtubule severing enzymes. These proteins belong to the AAX family of proteins, e.g., katanin is one of these enzymes. Katanin was the first microtubule severing enzyme identified. The catalytic subunit of Katanin, p60, has a role in cell division, cell motility, and regulated microtubule length and dynamics in mitotic and interphase cells. Other severing enzyme families include spastin and fidgetin. Fidgetin has yet to be proven to be a Severing enzyme in vitro. We performed the first single molecule characterization of katanin and fidgetin in vitro. We find that both katanin and fidgetin depolymerize microtubules at low concentration, but sever at high concentration. The depolymerization is faster from the plus end for katanin, but from the minus end for fidgetin. Using single molecule imaging, we find that, oligomerization, binding and diffusion depends on p60 nucleotide state. In contrast with katanin, fidgetin severing preferentially on GMPPCP-tubulin compared to GDP-tubulin. Finally, fidgetin presumably removes extended regions of protofilaments from the microtubule without cutting entirely through the microtubule, an ability called "protofilaments stripping." Both katanin and fidgetin are capable of severing microtubules, but their biophysical abilities and locations on microtubules are distinct from each other.

2441-Pos Board B427
A New Microtubule Gliding Assay Analysis of Microtubule Persistence Length
Lu Yu, Brian L. Van Hoozen, Carol E. Bodnar, Douglas S. Martin.
Microtubules are cytoskeletal polymers which play a role in cell division, cell mechanics, and intracellular transport. Each of these functions requires microtubules that are stiff and straight enough to span a significant fraction of the cell diameter. As a result, the microtubule persistence length, a measure of stiffness, has been actively studied for the past 15 years. Curiously, short microtubules are 10-50 times less stiff than long microtubules. In order to study this phenomenon, we have developed a new kinesin-driven microtubule gliding assay analysis of persistence length. By combining sparse fluorescent labeling of individual microtubules with single particle tracking of individual fluorophores, we tracked microtubule gliding trajectories with nanometer-level precision. By varying the surface density of kinesin, we measured the persistence length of microtubules on length scales from 100s of nanometers to 10s of micrometers. Microtubule persistence length increased from about 200 micrometers to 3 mm across these scales, consistent with previous experiments at short or long length scales. The scale dependence of persistence length we measured supports models in which protofilaments are loosely coupled near microtubule tips, becoming tightly coupled far from the tip.
cardiac muscle. The microtubule must be rigid enough to structurally support the cell, yet dynamic to reorganize during mitosis. We are investigating the effect of "old age" on microtubule rigidity using freely fluctuating taxol-stabilized, fluorescent microtubules in vitro. We find that the persistence length does not depend on the contour length when the measurements are all taken within several hours, but the persistence length does change on the order of a day. We also find that the noise floor is higher for new (within hours of polymerization) microtubules, perhaps due to the presence of unpolymerized dimers. After 24 hours, the noise decreases and the data is the most reproducible. After 48 hours, the noise rises again, likely due to disintegration of old microtubules and aggregation of dimers. We have also tested the effects of tubulin type (bovine and porcine) and rhodamine content on the persistence length value and the error in the measurement.

2446-Pos Board B432
Dynamic Instability of Microtubules: The Role of Topological Phonon Modes
Camelia Prodan, Emil V. Prodan, Sandhya Venkataraman, Enas Shehadeh.
Dynamic instability and the action of taxol on microtubules are yet not understood. However microtubules and dynamic instability are one of the best targets for cancer drugs. The main idea of the presentation is that the defects of the microtubules may display remarkable properties called topological phonon states. Recently, a new class of materials, topological insulators, has been discovered. These materials are insulators in bulk but have the extraordinary property that, if an edge is cut, electronic states appears at the edge. We advanced the idea that MTs have similar properties but with phonon states rather than electronic. This edge states are consequences of the unusual bulk properties of the microtubules, they cannot be destroyed by any chemical, mechanical etc treatment of the edge and they play a role in DI. Taxol may change the bulk properties of the MTs thus changing the edge states.

2447-Pos Board B433
Cdk1 (Cdc28) Phosphorylation of Gamma-Tubulin Couples Spindle Microtubule Dynamics to the Metaphase-Anaphase Transition
Elena Nazarova, Daici Chen, Jamie M. Keck, Michele H. Jones, Catherine Wong, John Yates III, Mark Winesy, Jackie Vogel.
Gamma-Tubulin is an evolutionarily conserved nucleator of microtubules. Gamma-Tubulin is a phospho-protein and the phosphorylation state of a conserved residue in the C-terminus (Y445) contributes to normal spindle function and actin-microtubule coupling in budding yeast. Here we report that gamma-tubulin is phosphorylated at S360, a Cdk1/Cdc28 site. Phosphorylation of S360 in vivo was global in a study of the phosphorylation of the spindle pole body. We confirmed Cdc28-Cib2 could phosphorylate S360 by in vitro kinase assay and peptide mass fingerprint by mass spectrometry, and in vivo using two dimensional-AGE. A phospho-mimetic mutation (tub4-S360D) causes mitotic delay but does not inhibit recruitment of the gamma-tubulin complex (reported by GRIP Spc97-EGFP) to spindle poles. Cytoplasmic microtubule function is normal in tub4-S360D cells but spindle microtubule function is altered. High-resolution analysis of spindle dynamics revealed fluctuations in length in metaphase and anaphase spindles. The velocities of spindle elongation in anaphase were similar in S360D mutant. We propose that S360 phosphorylation of gamma-tubulin by Cdk1 may play an important role in the control of spindle microtubule dynamics during the metaphase-anaphase transition.

Cardiac Muscle II

2448-Pos Board B434
The Effect of Dilated Cardiomyopathy (DCM) Mutations of Tropomyosin on Force Generation and Cross-Bridge Kinetics in Thin-Filament Reconstituted Bovine Cardiac Muscle Fibers
Fan Bai, Heather Groth, James Potter, Masataka Kawai.
Two DCM mutations (E40K and E45K) of Tropomyosin (Tm) were examined using the thin-filament extraction and reconstitution technique. Effects of Ca2+ on the G and P sites were studied at 25°C and compared to WT. Both E40K and E45K showed significantly lower high-Ca (pCa 4.66) tension (Tmec, 1.21 ± 0.06 and 1.24 ± 0.07, respectively), low-Ca (pCa 7.0) tension (Tmec, 0.07 ± 0.02 and 0.06 ± 0.02 at pCa 7.0), and Ca2+ active tension (Tmax = Tmec + Tic, 1.15 ± 0.08 and 1.08 ± 0.06, respectively) compared to WT by using taxol-stabilized, fluorescent microtubules in vitro. All tensions were normalized to Tmec in the tension of actin-filament reconstituted myoscardium at the standard activating condition. P0Ca (Ca2+ sensitivity) of E40K (5.23 ± 0.02) and E45K (5.24 ± 0.03) was similar to WT (5.26 ± 0.03). The cooperativity increased in both mutants (E40K 3.24 ± 0.29, E45K 3.73 ± 0.25) compared to WT (2.80 ± 0.17). Four equilibrium constants were deduced using sinusoidal analysis. E40K and E45K showed significantly increased K0 (ATP association constant, 5.21 ± 0.40 mM−1 and 1.28 ± 0.26 mM−1, respectively) than WT (0.82 ± 0.15 mM−1). E40K also showed significantly lower V0 (force generation step: 0.78 ± 0.12) and K0 (PI association constant: 0.18 ± 0.03) than E45K (1.52 ± 0.21 and 0.28 ± 0.04, respectively) and WT (2.35 ± 0.43 and 0.39 ± 0.07). The cross-bridge distribution of the three Tms was calculated from the equilibrium constants. E40K showed >20% less force generating cross-bridges than WT, but E45K remained similar to WT. These results indicate that, under pCa 4.66, force/cross-bridge is >20% less in E45K than WT, but remains similar in E40K. We conclude that the decreased force generating ability of these mutants may be the underlying cause of the pathogenesis of DCM.

2449-Pos Board B435
Ocult Myopathic Effects of K118C Mutation in Cardiac Tropomyosin 1 Detected in Isolated and Cultured Adult Mouse Cardiomyocyte
Hongguang Wei, J.-P. Jin.
Isolated adult cardiomyocytes provide a valuable system for cellular level phenotype studies. However, an N-terminal truncation of cardiac troponin T (cTnT-ND) found in ischemia-reperfusion with functional consequences also occurs during the perfusion-based cell isolation. Here we utilized short-term culture to allow isolated cardiomyocytes to recover for the study of a cardiac tropomyosin 1 mutation (cTnT-K118C). Adult cardiomyocytes were isolated from wild type and cTnT-K118C transgenic mice. Myofilament proteins were monitored using SDS-PAGE. Western blot and Pro-Q phosphoprotein staining. Paced contraction was examined on day 0 and day 2 of culture using edge detection. The cultured adult mouse cardiomyocytes maintained normal content of myosin, tropomyosin, cTnT and cTnI in culture for over 4 days. cTnT-ND declined from ~7.7% of total cTnT at day 0 to ~2.9% after 2 days of culture. The PKA-dependent phosphorylation of cTnI was preserved in 2 day-cultured cells. The amplitude of shortening in wild type myocytes on day 0 and day 2 were 3.05 ± 0.53% and 3.79 ± 0.68%, respectively, with statistical difference. The amplitude of shortening and the velocities of shortening/re-lengthening were decreased in day 0 and 2-day-cultured cTnT-K118C cardiomyocytes as compared with wild type controls, but only the 2-day-cultured group showed statistical significance (P = 0.05). The slack length of the cardiomyocytes was 11.9 ± 2.0 μm for wild type and 146.1 ± 2.9 μm for cTnT-K118C groups (P < 0.01) with no increase in sarcomere length or cell diameter. The data demonstrated that 2-day-cultured adult mouse cardiomyocytes were effectively recovering from the proteolytic modification of cTnT induced during isolation while maintaining the original level of contractility. The study of isolated cardiomyocytes from young adult transgenic mice without heart failure revealed that cTnT-K118C decreased contractility and caused cellular remodeling toward dilated cardiomyopathy.

2450-Pos Board B436
Increased Cardiac O-GlcNAc Transferase and O-GlcNAcase Association to Actin, Tropomyosin and ML1 in Diabetes: A Mechanism for O-GlcNAc Mediated Myofilament Calcium Desensitization
Genaro A. Ramirez-Correa, Chad Slawson, Wei D. Gao, Gerald W. Hart, Anne M. Murphy.
We demonstrated that normal cardiac myofilaments contain 32 total O-GlcNAcylation sites on HcH, Actin, Tropomyosin and ML1 in Diabetes Mellitus (DM) models. Yet, the mechanisms of O-GlcNAc-induced myofilament Ca2+ desensitization remain unclear. We investigated the effect of O-GlcNAc removal by an engineered hexosaminidase (CPJ) on Ca2+ sensitivity of skinned hearts of control and DM type 1 rats. We found that 1 hour exposure to CPJ reversed myofilament Ca2+ desensitization in DM cardiac muscles (EC50 8.17 ± 0.48 μM p<CPJ vs 2.73 ± 0.22 μM post-CPJ, n=5 vs n=4, P<0.029 ), but had no effect in control muscles (EC50 2.73 ± 0.17 μM p<CPJ vs 2.6 ± 0.15 μM post-CPJ, n=6 vs n=5, p<0.8). CPJ activity against O-GlcNAc was verified by western blot on treated lysates. These results suggest that in diabetic muscle O-GlcNAcylated directly affects myofilament Ca2+ sensitivity. To address potential mechanism(s), we characterized O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) protein interactions in fresh whole heart homogenates from both groups by immunoprecipitation followed by Western blotting. Rats were sacrificed at 5 °C and 24 h post mortality (t=24 h). The O-GlcNAc transferase was fractioned into two fractions, of which 80% ± 7% O-GlcNAc-induced cTnI cross-linking. In these fractions, we observed CPJ at 5 and 24 h significantly higher O-GlcNAc content of O-GlcNAc Transferase and O-GlcNAcase association in both control and Ac (n=4 vs n=4, p<0.01). These results strongly suggest that in DM a specific increase in O-GlcNAcylated myofilament proteins, and in OGT/OGA association with actin, tropomyosin and ML1 leads to dysfunctional regulation of myocardial contractility.