cell division. The microtubule must be rigid enough to structurally support the cell, yet dynamic to reorganize during mitosis. We are investigating the effect of "tub360D" on microtubule dynamics and Ca2+ sensitization using freely migrating thin-filament extracted reconstituted 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.

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 edge 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 they 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 or 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 Winey, 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 identified in a global analysis of the phosphoproteome of the spindle pole body. We confirmed Cdk2-Cdc2 could phosphorylate S360 in vivo by in vitro kinase assay and peptide identification by mass spectrometry, and in vivo using two dimensional-PAGE. 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 and wild-type spindles, but the initial phase of rapid elongation in anaphase spindles. The velocities of spindle elongation in anaphase were similar in S360D and wild-type spindles, but the initial phase of rapid elongation in anaphase spindles. We propose that S360 phosphorylation is important for the control of spindle microtubule dynamics during the metaphase-anaphase transition.

Cardiac Muscle II

Two DCM mutations (E40K and E54K) of Tropomyosin (Tm) were examined using the thin-filament extraction and reconstitution technique. Effects of Ca2+ and ATP, phosphate and ADP concentrations on force and the transients were studied at 25°C and compared to WT. Both E40K and E54K showed significantly lower high-Ca (pCa 4.66) tension (T HC, 1.21 ± 0.06 mN/m2), E40K and E54K showed significantly lower high-Ca (pCa 4.66) tension (T HC, 1.21 ± 0.06 mN/m2) compared to WT using freely migrating thin-filament stained 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.

2449-Pos Board B435 Occult Myopathic Effects of K118c Mutation in Cardiac Troponin I Detected in Isolated and Cultured Adult Mouse Cardiomyocyte Honhong 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 troponin I mutation (sTnl-K118C). Adult cardiomyocytes were isolated from wild type and cTnl-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 contents 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, without statistical difference. The amplitude of shortening and the velocities of shortening/re-lengthening were decreased in day 0 and 2-day-cultured sTnl-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 119 ± 2.5 μm for wild type and 146.1 ± 2.9 μm for cTnl-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 cTnI induced during isolation while maintaining the original level of contractility. The study of isolated cardiomyocytes from young adult transgenic mice with heart failure revealed that cTnl-118C 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 MLC 1 in Diabetes: A Mechanism for O-GlcNAc Mediated Myofilament Calcium Desensitization Genaro A. Ramirez-Correa, Chad Clawson, Wei D. Gao, Gerald W. Hart, Anne M. Murphy.
We demonstrated that normal cardiac myofilaments contain 32 total GlcNAc modification sites on MHC, Actin, MLC1, MLC2 and TnI and that exposure of skinned muscles to GlcNAc induces myofilament 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. Reduced interactions were observed between OGT and OGA proteins in diabetes. The OGT and OGA interactions with members of thick filaments were observed in diabetes. Yet, the mechanisms of O-GlcNAc-induced myofilament sensitivity remain unclear. We investigated the effect of O-GlcNAc removal by an engineered hexosaminidase (CPJ) on Ca2+ sensitivity of skinned muscles from 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 4.17 ± 0.08 mM vs 4.71 ± 0.11 mM, P < 0.05). The slack length of the cardiomyocytes was 119.4 ± 2.9 μm for wild type and 146.1 ± 2.9 μm for cTnl-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 cTnI induced during isolation while maintaining the original level of contractility. The study of isolated cardiomyocytes from young adult transgenic mice with heart failure revealed that cTnl-118C decreased contractility and caused cellular remodeling toward dilated cardiomyopathy.