analyzed by imaging rhodamine-labeled microtubules that were sampled by hot-spots to provide sub-diffraction limited images at 7nm resolution in the direction of movement and 135nm orthogonally. Degradation of signal-to-noise ratio due to side lobe modes was measured to be 6-dB. Intensity distribution generated by the NLS was measured to be broader than that of conventional imaging, which is consistent with the enhancement of imaging resolution. We have also studied correlation analysis between neighboring nanoantennas. This proves the possibility of measuring microtubular transport dynamics. NLS can be useful for moving objects that have a high labeling density or for performing fluctuation spectroscopy in small volumes and may allow ‘super-resolution on demand’ by customizing nanostructures.

3585-Pos Board B419
Loop 8 Plays a Role in Controlling S. cerevisiae Kinesin-5 Cin8 Motility and Function
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Microtubules Search for Lost Kinetochores by Pivoting Around the Spindle Pole
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During cell division, proper segregation of genetic material between the two daughter cells requires that the spindle microtubules attach to the chromosomes via kinetochores, protein complexes on the chromosome. In fission yeast, kinesin-8 capture by microtubules can be observed when kinetochores are lost in the nucleoplasm, which can be induced by spindle disassembly during metaphase. It is, however, unknown how microtubules find lost kinetochores. We observed that lost kinetochores are captured by microtubules pivoting around the spindle pole body, instead of extending towards the kinetochores. By introducing a yeast kinesin-8 C-terminal tail of Kip3, we showed that the observed random movement of microtubules is sufficient to explain the process of kinetochore capture. We thus reveal a mechanism where microtubules explore space by pivoting, as they search for intracellular targets.

3559-Pos Board B420
Microtubules Search for Lost Kinetochores by Pivoting Around the Spindle Pole

3560-Pos Board B421
Novel Roles of Kinesin-8 in Organizing Mitotic Spindles
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The kinesin-8 family of microtubule motors plays a critical role in microtubule length control in cells. The budding yeast kinesin-8 Kip3 is a microtubule plus-end-specific depolymerase, which apparently destabilizes microtubules and mitotic spindles. We identified a secondary tubulin-binding domain on the C-terminal tail of Kip3. With the tail-binding to tubulin, Kip3 can transport tubulin dimers along microtubules. Kip3 can also slide apart anti-parallel microtubules whereas parallel microtubules display a tug-of-war behavior in the presence of Kip3. To investigate the physiological role of this newly found microtubule-crosslinking activity of Kip3, we made a tail-less mutant form of Kip3, which does not contain the crosslinking activity. We expressed this mutant at the depolymerase activity similar to the wild-type Kip3. We found spindles are fragile and prematurely broke during anaphase. On the other hand, cells expressing a Kip3 mutant that loses the depolymerase activity but maintains motility and the crosslinking-activity, have more stabilized spindles, compared to kip3-null cells. These data suggest that Kip3 has a tail-mediated stabilizing effect on mitotic spindles. Combining the previously found depolymerase activity, we propose a ‘slide, crosslink and chew’ model to describe the roles of Kip3-kinesin-8 in organizing mitotic spindles.

3561-Pos Board B422
Gamma Tubulin Phospho Regulation: Insights into Spindle Assembly
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The mitotic spindle is composed of spindle poles, chromatin, and pole-to-pole and pole-kinetochore microtubules. Pole-kinetochore microtubules are anchored at the spindle poles, with the opposite end attached to centromeric chromatin. Pole-to-pole microtubules are represented by cross-linked microtubules from the opposite poles, and form the microtubule core bundle (CMB), that plays an important role in stability of pre-anaphase mitotic spindle. The mitotic spindle is a highly coupled mechanical system, whose dynamic behavior changes in response of Cdk1-dependent signaling.

In cells, the majority of spindle microtubules are assembled from nucleation sites located at the spindle poles, centrosomes in animal cells and spindle pole bodies in yeast. γ-tubulin is an essential part of γ-TuRC (γ-Tubulin Ring Complex), a template for nucleating the majority of microtubules in the cell. Here, we show the coupling of Cdk1-phosphorylation state of γ-tubulin with microtubule organization during assembly of the mitotic spindle. We use confocal microscopy and high-resolution analysis of fluorescently labeled proteins to characterize in vivo the dynamic behavior of the spindle during its assembly in wild-type cells. γ-tubulin is a key regulator of number and organization of microtubules. Here, we propose a model, where the phosphorylation state of γ-tubulin contributes to determination of microtubule fate during spindle assembly and stabilizing the spindle prior and during anaphase.

3562-Pos Board B423
Live Cell Imaging of the Human Cells Depleted with Kinesin Family Member C1 (KIFC1) and Stathmin/Oip18 shows that these Cells Process Mitosis with Lagging Chromosome and Micronuclei, Suggesting a Critical Role of KIFC1 and Stathmin/Oip18 in Genomic Stability during Mitosis
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The kinesin superfamily proteins (KIFs) take part in chromosomal and spindle movements during mitosis and meiosis. Most KIFs have plus-end directed motility. However, the kinesin family member C1 (KIFC1) has a catalytic core at the C-terminus and has minus-end directed motility (C-type motors). In this study, we examined the function of KIFC1 during mitosis using live cell imaging. The number of cells with multiple microtubule organizing centers were increased when KIFC1 protein level was decreased by small interfering RNA in the primary IMR90 cell. Live cell images also confirmed that cell cycle was delayed in early mitosis and multiple poles were induced when KIFC1 was knocked down by lentiviral shRNA in IMR90 cells. MDA-MB-231 breast cancer cells with increased multiple poles by siRNA of KIFC1 managed to recluster the multiple poles to pseudo-bipolar to finish mitosis when observed using timelapse microscopy. KIFC1 depletion also increases micronuclei and lagging...
chromosomes not only in cancer cells but also in primary IMR90 cells, which are possible causes for genetic instability. Taken together, these observations suggest that KIFC1 is essential for proper chromosome segregation to maintain genomic stability. Yeast two-hybrid search and co-immunoprecipitation of KIFC1 demonstrated that KIFC1 directly interacts with Op18/shatamin, which regulates microtubule dynamics by inhibiting tubulin polymerization. When KIFC1 and Op18 were doubly knocked down in MDA-MB-231 cells, the portion of cells with multiple poles and the number of micronuclei was highly increased. These results suggest that KIFC1 functions with Op18 during mitosis.

3565-Pos Board B424
Mitotic Kinesin CENP-E is a Robust Tracker of Dynamic Microtubule ends
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Accurate chromosome segregation during mitosis requires durable linking between kinetochores and plus ends of spindle microtubules. During metaphase the ends of kinetochore microtubules continuously assemble and disassemble but the chromosomes remain stably attached and move concomitantly with microtubule dynamics. So far only Dam1 kinetochore complex has been reported to be able to maintain stable association with dynamic microtubule ends in vitro. The homologous genes, however, are present only in fungi, so the molecular identities of kinetochore-microtubule couplers in other cells are not known. Here, we report that a conserved kinesin-like CENP-E, playing an essential role in chromosome segregation, can maintain long-lasting association with the ends of dynamic microtubules in vitro and couple microtubule depolymerization to the motion of a microtubule cargo. Using purified recombinant CENP-E from Xenopus laevis we show that a truncated dimeric motor, which lacks a stalk and a tail, fails to track the dynamic microtubule ends. However, the rate and processivity of the plus-end-directed motility and the response to applied force are similar for the full length and truncated proteins. Interestingly, a walking full length CENP-E has a folded configuration and remains compact even under tension, suggesting that such conformation is important for CENP-E's functioning at the kinetochore. To examine whether the 230nm-long CENP-E stalk is important for kinetochore-microtubule interactions, we used siRNA-mediated depletion of endogenous CENP-E in cells with stable expression of a truncated "bonsai" version of CENP-E, which localizes normally to kinetochores. In cells with "bonsai" CENP-E the chromosome congression was delayed. Furthermore, the aligned chromosomes showed marked instability and quickly moved away from the metaphase plate, implying defects in kinetochore-microtubule attachment. We propose that CENP-E exerts some of its mitotic functions via its non-motor domains, which enable it to track dynamic microtubule ends.

3565-Pos Board B425
Dynnein Function in T Cell MTOC Translocation and Vesicle Movements
Laura Christian1, Kathryn Chin1, Amanda Siglin2, John Williamson3, Dynein Function in T Cell MTOC Translocation and Vesicle Movements

3565-Pos Board B426
Analysis of E-Cadherin as a Tension Sensor at Epithelial Cell-Cell Junctions
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Cells are mechanosensitive systems in which the detection of external mechanical forces by proteins that span the plasma membrane leads to changes in cell organization, differentiation and proliferation. Cell-cell contacts are thought to be sites of mechano-transduction, and a major cell adhesion protein E-cadherin is a candidate protein to transduce forces between cells under tension. To determine whether force transduction occurs through E-cadherin, we inserted a force sensor module into the E-cadherin cytoplasmic domain. The force sensor (TSMod) is a 32-aa-long flexible peptide capped by mTFP and Venus that together form a FRET pair. FRET efficiency scales inversely with linker extension, and thus reports the tension exerted on the sensor within the host protein. Using FRET intensity ratio imaging in live MDCK cells, we show that the E-cadherin construct is recruited to cell-cell contacts like its endogenous counterpart. We observed decreased FRET both at cell-cell contacts and at the plasma membrane distant from intercellular junctions, indicating that the E-cadherin cytoplasmic domain is under tension regardless of its recruitment to intercellular contacts. Significantly, we observed increased FRET upon actin cytoskeleton disruption or depletion of alpha-catenin, a putative linker between actin and E-cadherin, regardless of subcellular location. These results indicate that the E-cadherin complex is under tension through the catenin complex and actin cytoskeleton, and that this is a constitutive state that is independent of cell-cell adhesion.

3566-Pos Board B427
Cells Sense Rigidity of their Micro-Environment by a Unique Force-Sensing Displacement Cassette
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Eukaryotic cells modulate their gene expression profile and differentiation in response to physical cues in their micro environment. The exact mechanism by which the cells sense the stiffness of the substrate has not yet been completely understood. Recent studies with micropillar arrays indicate that cells move fibronectin pillars for a constant displacement irrespective of the stiffness of the substrate. However, the exact mechanism by which the cells sense the stiffness of the substrate has not yet been completely understood. Recent studies with micropillar arrays indicate that cells move fibronectin pillars for a constant displacement irrespective of the stiffness. Thus, rigidity sensing may involve the integration of the force required. To further understand the mechanism involved, we increased the center-to-center spacing of the micropillars to 3 micrometers and compared two different cell lines. We observed that the displacements of the pillars were constant over an order of magnitude variation in stiffness of pillars and a six-fold variation in spacing; however, the mouse embryonic fibroblast (RPTPζ ÷÷/-) and cells foreskin fibroblasts (HFF) cells exerted consistent peak pillar displacements of ~70nm and ~120nm respectively. Chemical and genetic perturbations of likely components of the mechanosensing machinery are being used to probe its composition and working. Delineating the components of this cassette is crucial to understanding the sensing of the substrate stiffness by cells with implications for cell differentiation and metastasis.

3567-Pos Board B428
Influence of Substrate Stiffness on Cell Spread Area
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It is known that various cell types can sense and respond to the mechanical properties of their microenvironment. Specifically, cells have been known to spread more when cultured on stiff substrates and are able to match their...