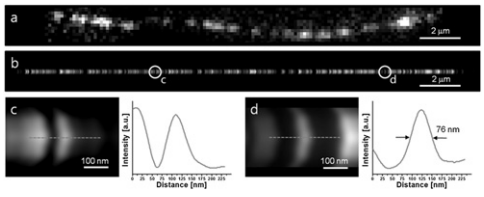
analyzed by imaging rhodamine-labeled microtubules that were sampled by hot-spots to provide sub-diffraction limited images at 76nm resolution in the direction of movement and 135nm orthogonally. Degradation of signal-to-noise ratio due to side lobe modes was measured to be 6-dB. Intensity distribution 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.



3558-Pos Board B419

Loop 8 Plays a Role in Controlling *S. Cerevisiae* Kinesin-5 Cin8 Motility and Function

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Kinesin-5 proteins are microtubule associated motors, which are highly conserved from yeast to human cells. They share high homology in their catalytic motor domain sequence, fulfill similar essential mitotic roles in spindle assembly and anaphase B spindle elongation and, until recently (*Roostalu et al., Science, 2011*), were all thought to move towards plus ends of microtubules. Mechanisms that regulate Kinesin-5 function, specifically during anaphase B, are not well understood.

S. cerevisiae cells express two Kinesin-5 homologues, Cin8 and Kip1, which overlap in function. Here we have examined *in vitro* and *in vivo* functions and regulation of Cin8 during anaphase B. We followed Cin8 localization and carried out single molecule fluorescence motility assays to study Cin8 motile properties. We found that *in vitro*, Cin8 molecules are able to switch directionality along a single microtubule as a function of ionic strength conditions and that during anaphase B, Cin8 moves not only towards the plus, but also towards the minus ends of spindle microtubules.

Compared to kinesin-5 homologues of higher eukaryotes, *S. cerevisiae* Cin8 carries a uniquely large insert in loop 8 in its motor domain. To probe the role of the large loop 8 in the directionality switch of Cin8, we studied a construct in which this segment was replaced with the seven amino acids of loop 8 in the related *S. cerevisiae* kinesin-5 Kip1 (Cin8Δ99) (*Hoyt et al.,J Cell Biol, 1992*). We examined the anaphase B localization and *in vitro* motile properties of the Cin8Δ99 variant. Using combined *in vitro* and *in vivo* approaches, we were able to characterize the role of loop 8 in controlling Cin8 motility and function during *S. cerevisiae* anaphase.

3559-Pos Board B420

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, kinetochore 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 theoretical model, we show 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.

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, phospho-mimicking and phosphor-inhibiting mutants. We reconstructed short (1-2.5 µm) spindles using EM tomography and could establish the relative stoichiometry of γ -TuRC : microtubules in phosphor-mutants of γ-tubulin. Our results suggest that 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.

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Live Cell Imaging of the Human Cells Depleted with Kinesin Family Member C1 (KIFC1) and Stathmin/Op18 shows that these Cells Process Mitosis with Lagging Chromosome and Micronuclei, Suggesting a Critical Role of KIFC1 and Stathmin/Op18 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 knockdowned 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-bipoles to finish mitosis when observed using timelaps microscopy. KIFC1 depletion also increases micronuclei and lagging