that CENP-E plays an important mitotic role at the kinetochore-associated microtubule tips. To determine the molecular mechanism of CENP-E tip-tracking, we characterized two purified recombinant fragments of CENP-E: one containing the motor and neck domains and the second with the dimeric C-terminal tails. The motor-containing truncated protein walked on the microtubule wall in essentially the same manner as the full length CENP-E, while the C-terminal tail exhibited rapid diffusion. Neither of these fragments showed the tip-tracking, however, this activity was recapitulated by artificially joining these two proteins by conjugating to Qdots. A computational model of CENP-E motility successfully described the tip-tracking ability by repeating the cycles of plus-end-directed walking and the tail-mediated diffusion of the microtubule wall-tethered motor. This novel ''tethered motor'' mechanism of tip-tracking does not rely on the specific properties of the assembling or disassembling microtubule tips, explaining why CENP-E can tip-track bi-directionally, i.e. with the growing and shortening microtubule ends. Together, these results establish the requirement for CENP-E in stably linking the kinetochores to dynamic microtubule tips, and provide a detailed molecular mechanism to explain how CENP-E can achieve this function.

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The Role of Kinesin-8 Proteins in Kinetochore Movements and Spindle Dynamics in Fission Yeast

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The fission yeast proteins Klp5p and Klp6p are plus-end directed motors of the kinesin-8 family known to promote microtubule depolymerization. During mitosis these motors localize to the mitotic spindle. Although the kinesin 8s are not essential for cell division, their deletion leads to elongated interphase and mitotic microtubules and a delayed anaphase onset. To understand kinesin-8 roles in kinetochore movements, we study cells carrying a cold-sensitive allele of beta-tubulin. Placing these cells at 16^{o}C for 7 hours depolymerizes their microtubules, arrests them in mitosis, and allows kinetochores to detach from the spindle poles. We then re-warm the cells and use fluorescence microscopy to observe chromosome reattachment and mitotic progression. We have tracked kinetochore and spindle pole positions in four dimensions, allowing us to quantify the dynamics of the kinetochore and spindle. Similar methods were previously used to characterize kinetochore dynamics in cells deleted for all the fission-yeast minus-end directed motors; there was no difference in the rate of final kinetochore-to-pole movements when these motors were removed. Our preliminary data show little difference in the rates of kinetochore-to-pole movements when the kinesin-8 genes are removed, but there are differences in kinetochore dynamics. These mutants show evidence of kinetochore-spindle association without the characteristic movement of the kinetochore toward the capturing spindle pole. In *klp5D* mutants some kinetochores are pushed as much as 4 spindle lengths away from either spindle pole, while *klp6D* mutants and wild type cells show no such behavior. Kinesin-8 deletion mutants also show increased spindle length fluctuations. In all these strains, most kinetochores are eventually recaptured and mitosis proceeds.

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Photocontrol of ATPase Avtivity of Kinesin Kif18A using Various Existing Azoboenzene Derivatives

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The shape and function of the mitotic spindle depends on the cooperation action of various kinesins. Recently, it was demonstrated that the kinesin Kif18A is a central component for the correct alignment of chromosomes at the spindle equator. BTB-1 inhibits ATPase activity of kif18A but not of other mitotic kinesins. BTB-1 is a potent inhibitor for kinesin Kif18A. In mitosis cell, inhibition of Kif18A by BTB-1 occur disorder chromosome congression and cell lead to apotosis. In vitro, BTB-1 inhibits the motility of Kif18A in a reversible manner. BTB-1 inhibits Kif18A ATPase activity in an ATP competitive manner but microtubule. BTB-1 is composed of chloro group and the nitro group and the two aromatic rings. Azobenzen is one of the photochromic molecules that change their shapes and properities drastically upon ultra-violet (UV) and visible (VIS) light irradiation. Previously, we have incorporated azobenzen derivative into functional region of ATP driven motor protein and succeeded to control microtubule dependent ATPase activity reversibly by UV-VIS light irradiation. Interestingly, BTB-1 shows structural similarity to azobenzen derivatives. In this study, we examined photo-reversible inhibitory effects of various existing azobenzen derivatives for the ATPase activity of kif18A. Cis isomer of 4-aminoazoboenzene-4-sulfonic acid induced by UV irradiation inhibited ATPase activity of Kif18A more effectively than its trans isomer. Other azobenzen derivate were also examined.

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Synthesis of Novel Photochromic Inhibitors of Mitotic Kinesins Eg5 and Kif18a and their Phtoresponsive Interaction with the Kinesins

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Kinesin Eg5, a member of the kinesin-5 family, is essential for formation of bipolar spindle during cell division. Kinesin Kif18a, a member of the kinesin-8 family, plays a role in chromosome congression. Eg5 and Kif18a is potential drug target for cancer chemotherapy. Monastrol and Strityl-L-cysteine (STLC) is potent inhibitor specific for kinesin Eg5. BTB-1 is potent inhibitor for kinesin KIf18a.

In this study, we have tried to design and synthesize various STLC and BTB-1 analogues composed of photochromic molecules in order to apply as photoresponsive inhibitors.

We used azobenzene as photochromic molecule. their photo-reversible inhibitory effects for Eg5 and Kif18a were examined upon ultra-violet (UV) and visible (VIS) light irradiation. STLC analogue was synthesized by coupling reaction of 4-phenylazophenyl maleimide or 4-phenylazophenyl iodoacetoaminde with SH-group of cysteine. BTB-1 analog was synthesized by adding chloro group and nitro group to azobenzene.

The ability of these compounds to inhibit kinesin activity has been investigated in vitro microtubule-stimulated ATPase activity. They showed reversible absorption spectral changes upon UV-VIS light irradiations suggesting the cis-trans isomerization of azobenzen moiety. Preliminary experiments revealed that the Microtubules dependent ATPase activity of Eg5 was inhibited by photochromic STLC analogue reversibly upon UV-VIS light irradiation. We also examined inhibitory effect of photochromic BTB-1 analog for Kif18a.

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Gene Regulation

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Systems-Level Analysis of Stem Cells in the Larval Stage of the Human Parasitic Flatworm Schistosoma

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This study combines quantitative biophysical approaches and functional genomic analysis to understand stem cell development in the human parasite Schistosoma mansoni. We anticipate these parasites will become a unique model system in which studies of fundamental stem cell biology have the potential to impact a devastating human disease. Schistosoma flatworms parasitize over 230 million people worldwide, causing schistosomiasis, a neglected tropical disease with a global socioeconomic burden nearly equivalent to that of HIV/AIDS and malaria. In addition to their relevance to human health, the complex biology of these parasites has fascinated scientists for nearly a century. Schistosomes transmit via a complex life cycle that alternates between obligate parasitism of snail intermediate and human definitive hosts. To facilitate these transitions, schistosomes develop sequentially four distinct body plans, a process accomplished by a population of stem cells that undergo multiple rounds of stage-specific self-renewal and differentiation. Here, we focus on the development of sporocysts (larval schistosomes) upon entry into snail hosts. At this stage, stem cells divide and asexually produce many simultaneously developing daughter embryos, leading to a geometric expansion of single sporocysts to thousands of infectious cercariae. Since little is known about the biology of these stem cells, we are developing novel fluorescence imaging methods to reconstruct the trajectories of these cells during development. We are integrating these studies with genome-wide analyses of gene expression and function. Our studies show that these cells resemble pluripotent stem cells called neoblasts, that drive long-term tissue homeostasis and regeneration in long-lived free-living flatworms. Functionally, we identify a group of evolutionarily conserved post-transcriptional regulators which maintain the enormous reproductive capacity of these cells.