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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Synthesis of Novel Photochromic Inhibitors of Mitotic Kinesins Eg5 and Kif18a and their Photoreceptive Interaction with the Kinesins
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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 iodoacetamidene 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 azobenzene moiety. Preliminary experiments revealed that the Microtubules dependent ATPase activity of Eg5 was inhibited by the 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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