Title

Regulation of Eg5 and TPX2 During Mammalian Mitosis

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Abstract

During mitosis, the microtubule cytoskeleton is completely rearranged to form a bipolar spindle that functions to congress and segregate a complete set of genetic material into two nascent daughter cells. The kinesin-5 family of molecular motor proteins is required for spindle pole separation in most organisms. By cross-linking and sliding apart antiparallel microtubules extending from opposite poles, Eg5, the human kinesin-5 family member, produces the outward force necessary to establish spindle bipolarity. Eg5 has recently been demonstrated to interact with the spindle assembly factor targeting protein for Xklp2, or TPX2. TPX2 contributes to many aspects of spindle assembly, including activating the mitotic kinase Aurora A, nucleating microtubules around chromosomes, and targeting several proteins to the spindle.

In this dissertation, I use in vitro experiments to explore the regulation of TPX2 and Eg5 and the physiological significance of their interaction. By assaying the activity of populations of Eg5 motors, I show that TPX2 inhibits Eg5-driven microtubule gliding and relative microtubule sliding; an interaction between TPX2 and Eg5 contributes to the inhibition of the motor. Using total internal reflection fluorescence (TIRF) microscopy, I show that Eg5 accumulates on microtubules in the presence of TPX2, but less in the presence of TPX2-710, a truncated TPX2 construct lacking the Eg5 binding domain. These results contribute to a model where, in vivo, TPX2 alters the activity of Eg5 on, and also localizes the motor to, spindle microtubules, to achieve spindle formation.

Using TIRF microscopy in live cells combined with automated particle tracking, I explore the dynamics of localization and purification (LAP)-tagged Eg5 punctae under two conditions: dynein inhibition and TPX2 knock-down. I show that on astral microtubules, dynein activity is required for the minus-end-directed movement of Eg5 punctae, and this movement is dependent on TPX2. On overlapping microtubules in the spindle midzone, the magnitude of the velocity of Eg5 is similar under all conditions tested, demonstrating that perturbations to Eg5 activity might have an effect on the overall structure of the spindle without affecting the dynamics of Eg5. These results contribute to a model where Eg5 is transported poleward on astral microtubules by dynein, in a TPX2-dependent manner, and that the dynamics of Eg5 punctae in the spindle midzone, but not

the spindle structure, are unaffected by dynein inhibition or the absence of TPX2.

I use in vitro assays with epifluorescence and TIRF microscopy to explore the binding dynamics of TPX2 on microtubules. TPX2 binds to both GTP-like and GDP-like microtubule lattices, in a concentration-dependent manner, and also exhibits microtubule binding activity within both of its N- and C- termini. The electrostatic interactions that TPX2 makes with microtubules are dampened by the addition of salt to the TPX2 binding assay, but binding does not require the negatively-charged tubulin E-hook. The dwell time of TPX2 when assayed by single molecule TIRF microscopy is 85 seconds on average, and some molecules of TPX2 exhibit bidirectional diffusion along the microtubule lattice.

When assayed by single molecule TIRF microscopy, TPX2 and TPX2-710 similarly inhibit fluorescently tagged Eg5 (Eg5-EGFP) motors, suggesting that TPX2 can act as a roadblock, or can sterically block Eg5 motors from translocating along a microtubule protofilament. An interaction between TPX2 and Eg5, however, contributes to enhanced inhibition of Eg5 motors, demonstrating that TPX2 is also a brake, or interacts specifically with Eg5 to tether the motor to the microtubule as a mode of inhibition. Adding an excess of a purified C-terminal construct of TPX2 comprised of the Eg5 binding domain, CT35, partially alleviates the inhibition of TPX2 on Eg5-EGFP motors in TIRF, but does not alleviate the inhibition of TPX2 on the microtubule-gliding activity of populations of dimeric Eg5 motors. A purified, truncated construct of TPX2, comprised of the N-terminal half of the protein sequence (GST-NT), inhibits the microtubule-gliding activity of populations of dimeric Eg5 motors, but not as strongly as full-length TPX2, further indicating that an interaction with Eg5 contributes to the braking effect of TPX2 on Eg5. Preliminary data shows that the dynactin subunit p150 speeds up Eg5-EGFP motor activity, but in the presence of both p150 and TPX2, Eg5- EGFP motor activity is inhibited. These results suggest that Eg5 could be differentially regulated by TPX2 and p150 - TPX2 acting as a brake and p150 acting as an accelerator.

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