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## Microtubule dynamics and behavior during the assembly and disassembly of mammalian mitotic spindles

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### Abstract

The mitotic apparatus is composed of a complex, seemingly chaotic, but wonderfully organized compilation of cytoskeletal elements and motor proteins. Its task is to assure that each daughter cell receives a single copy of the genome. Microtubules are the biological polymers that make up the bipolar mitotic spindle, serving as tracks to separate sister-chromatids to opposite sides of the dividing cell. Understanding the behavior of microtubules during mitotic entry and exit is extremely important for diagnosing possible defects of atypical or failed mitosis. ^ This research uses the advantages of GFP technology to investigate the dynamics of microtubules throughout the cell cycle. Cells permanently expressing GFP- $\alpha$ -tubulin were generated using lipid transfection and drug selection. We show that GFP-tubulin had little effect on the behavior and role of microtubules; therefore, we conclude that cells expressing GFP- $\alpha$ -tubulin are a reliable method for studying microtubule behavior. ^ Live imaging of an epithelial cell line (LLCPK1) stably expressing GFP- $\alpha$ -tubulin revealed a microtubule bundling phenomenon that stabilizes microtubules during prometaphase. Further analysis shows that these bundles are pulled into the forming spindle in a dynein dependent manner. Our final model is an extension of the search and capture model, where we suggest that centrosomal microtubules also capture peripheral microtubule bundles during spindle assembly. ^ During mitotic exit, the process of moving microtubules inward is reversed, as microtubules are subject to outward forces during anaphase. Three main events are documented following anaphase onset: microtubule elongation, microtubule release, and spindle pole fragmentation. These events serve to populate regions of the cell that were devoid of microtubules during metaphase. Evidence is also presented in support of how microtubules

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might specify the location of the contractile ring. We show that microtubules at the equator target the cortex more frequently than elsewhere in the cell, suggesting a faster rate of delivering furrow-initiation factors. ^ We use numerous GFP fusion proteins that allowed us to tackle our biological question from several angles, which wouldn't have been possible to answer otherwise. In summary, my work provides new insight into the behavior of microtubules during the assembly and disassembly of mitotic spindles in mammalian somatic cells. ^

## Subject Area

Biology, Genetics|Biology, Cell

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