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Total Internal Reflection Fluorescence Microscopy to Study Microtubule Dynamics

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Microtubules are cytoskeletal filaments inside cells with essential roles for cell morphology, intracellular transport, and mitosis. The heterodimer, made from alpha-beta tubulin, is the basic subunit of the microtubule lattice. These dimers associate longitudinally to form protofilaments and laterally to create sheets that roll into a tube, typically with 13 protofilaments *in vivo*. Since dimers always associate in the same direction, the entire microtubule filament is polarized with alpha at the minus end and beta at the plus end. Microtubules have an intrinsic ability to polymerize (grow) and depolymerize (shrink) with stochastic switching between these two states termed catastrophes (growing to shrinking) and rescues (shrinking to growing). This process is termed "dynamic instability" and it can be recapitulated *in vitro* (1). In cells, microtubules also display dynamic instability, but the dynamics are finely tuned depending on the cell type and the location within the cell. MAPs are responsible for enhancing these dynamics by nucleating, promoting polymerization, stabilizing, or destabilizing microtubules (2-13).

We are interested in recapitulating the dynamics observed in cells by systematically adding stabilizing and destabilizing microtubule-associated proteins. One interesting set of microtubule stabilizing proteins is the Ending Binding (EB) proteins. EB was discovered as a protein that binds to adenomatous polyposis coli (APC) tumor suppressor protein (13). EB proteins can bind to (2) and are enriched at the tips of microtubules (14,15). Plus-tip tracking has been recapitulated *in vitro* using the yeast homolog, Mal3 (16), and with mammalian EB proteins (3,17). Both in cells and *in vitro*, it has been demonstrated that EB proteins bind transiently to the end and turn over rapidly.

We are also interested in the effects of destabilizing microtubule-associated proteins, such as the microtubule-severing enzymes. Microtubule severing enzymes are a novel class of MAPs that use ATP to remove tubulin subunits from the microtubule. They can remove tubulin from the middle of the microtubule, called severing. They can also remove tubulin from the ends, appearing as depolymerization (18). With these two modes of operation, microtubule-severing enzymes would have the unique ability to compete with stabilizing MAPs microtubules to control the dynamic instability of microtubules.

In order to measure the dynamics while observing the binding of associated proteins, we will use two-color total internal reflection fluorescence (TIRF) microscopy on purified system. We systematically alter the concentrations of stabilizing and destabilizing proteins to examine the effects on microtubule dynamics. We can recapitulate the dynamics of microtubules observed in cells by controlling the activity of stabilizing and destabilizing microtubule-associated proteins.

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