Abstract

The cytoskeleton provides living organisms with machinery to move. On the molecular scale, the same cytoskeletal components undergo constant reorganization to contribute to distinct cellular processes, such as the navigation of neuronal growth cones in brain development or the creation of chiral actin flows during cellular division.

During brain development, neuronal growth cones navigate by employing cytoskeletal crosstalk between actin filament networks and microtubules. Crosstalk in the growth cones between these cytoskeletal components was linked with known microtubule polymerase human cytoskeleton associated protein 5 (CKAP5; homolog of XMAP215, MSPS, Zyg9), while the details of the direct molecular mechanism of CKAP5-provided remodeling of both microtubules and actin networks are lacking. Therefore, we used total internal reflection fluorescence (TIRF) microscopy together with state-of-the-art *in vitro* reconstituted assays combining microtubules, actin filament networks, and recombinant proteins to understand cytoskeleton-provided molecular mechanisms underlying cellular motion. In this thesis, we show that CKAP5 alone bundles both supposedly randomly oriented and parallel actin filaments, crosslinks actin filaments to microtubules regardless of their polarity, positions prevailing actin bundles along patterns provided by microtubules, and enables tip-tracking of dynamic microtubules along actin filament bundles.

Furthermore, the actomyosin cortex located below the cellular membrane is constantly and dynamically remodeled to create chiral actin flows that sequentially lead to counter-rotation of the opposing halves in dividing embryos and establishment of the left-right body asymmetry. Proteins associated with actin filaments (myosins and formins) have been linked with symmetry breaking with limited insight into direct molecular mechanisms. Using TIRF microscopy and *in vitro* reconstitution, we show that clustering of formins by myosins is insufficient for the chirality of centered actin networks, and bundling does not play a role in this simplified system. At the same time, friction between growing actin filaments and surface induces chirality, while we observed chiral bias in a counterclockwise direction.

Lastly, this thesis briefly discusses a general advance in methodology that aims to slightly ease the preparation phase of the *in vitro* reconstitution assays that use fluorescence microscopy to visualize the studied system.

Key words: actin filaments, microtubules, cytoskeletal-crosstalk, CKAP5/XMAP215, chiral actin flows