Understanding the Tip Tracking Mechanism of End-Binding Proteins on Microtubules

Abstract

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Microtubules are dynamic cytoskeletal structures that act as tracks for the long-distance transport of cargo by motor proteins inside cells. It has been shown that specialised end-binding proteins (EBs) preferentially bind to the specific conformation of the GTP-bound tubulin cap at the growing microtubule tip [1, 2]. Transient binding between cargo (such as vesicles) and these EBs can facilitate the directed, intracellular transport of the cargo without the use of any associated motor proteins. The exact biophysical mechanism behind this tip-tracking process has not yet been identified, and both the processes of EB-microtubule binding and cargo-EB binding must be understood separately before a complete model of the phenomenon can be produced. Here, we present analytical and experimental results that aim to elucidate the EB-microtubule binding mechanism. Our results suggest that increased EB dwell times at the microtubule tip are due the presence of both a 'weakly' and a 'fully' bound state.

The binding dynamics of the EB-microtubule interaction can be expressed as a pair of coupled, one-dimensional Fokker-Planck equations, from which it is possible to derive analytical forms of the average dwell time, effective diffusivity, and stationary-state distribution of the bound EBs. Our model predicts that a system with two distinct bound states will have two characteristic unbinding timescales. In contrast with previously published data [1, 2], our single-molecule experiments have generated a dwell time distribution that clearly displays these two timescales. Further comparison between analytical and experimental results suggests that a tip-localised increase in the transition rate into the fully bound state is responsible for the increased dwell time at the microtubule tip. Experiments involving monomeric EB constructs have been carried out in order to clarify whether the two detected bound states are the result of an intermediate electrostatic bound state, or whether each of the two binding sites on a single EB can bind separately to a microtubule.

Our analytical model has also been extended to study the binding dynamics of cargo permanently engaged with multiple EBs. For small numbers of cargo-bound EBs the composite system exhibits a dwell time distribution that is heavily dependent on the initial binding state of the system, but as the number of cargo-bound EBs increases the dwell time grows exponentially.

References

[1] P. Bieling et al. Clip-170 tracks growing microtubule ends by dynamically recognizing composite eb1/tubulin-binding sites. *J. Cell Biol.*, 183:1223–1233, 2008.

[2] S. M. Gouveia. In vitro reconstitution of the functional interplay between mcak and eb3 at microtubule plus ends. *Curr. Biol.*, 20:1717–1722, 2010.