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Cell Biology: Kinesin-14 Backsteps to Organize Polymerizing Microtubules

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Microtubules nucleated from an organizing center grow radially in all directions. A new study shows that, to organize those microtubules into arrays of parallel bundles, the kinesin-14 Cik1–Kar3 guides growing microtubule tips along pre-existing microtubules.

The organization of microtubules into parallel bundles is important for diverse intracellular processes, for example the polarization of epithelial cells, cell migration, chromosome segregation and neuronal transport. Microtubules undergo GTP-driven dynamic instability — stochastically switching between phases of polymerization and depolymerization — a process which is tightly regulated by microtubule associated proteins. Often, microtubules grow individually with their plus-tips directed outwards from a single microtubule-organizing center radiating in all directions, thus forming asters. Additionally to microtubule dynamics, molecular motor proteins can rearrange already existing microtubules.

Molecular motors are enzymes that consume chemical energy derived from the hydrolysis of ATP to generate directed forces. Some molecular motors (for example the members of the kinesin-14 family) can bind to two microtubules simultaneously, localizing into regions of microtubule overlap and thereby crosslinking the two microtubules. By moving directionally towards the minus-tips of the microtubules, kinesin-14 motors can generate forces between pairs of microtubules. This mechanism results in sliding of antiparallel microtubule pairs relative to each other and stable bundling of parallel microtubule pairs, thus sorting the pre-existing microtubules [1,2].

In a study published recently in *Cell*, Molodtsov *et al.* [3] describe a mechanism of microtubule organization fundamentally different from the motor-driven sorting described above. The newly discovered mechanism is dependent on both the dynamics of microtubules and the action of molecular motors. Using a broad range of experimental approaches ranging from *in vitro* single-molecule experiments to live-cell imaging, Molodtsov *et al.* show that a two-protein system can guide the tips of polymerizing microtubules along pre-existing microtubules. The protein complex comprises a microtubule tip-tracking protein of the end-binding EB1 protein family and the Cik1–Kar3 heterodimeric kinesin-14 from budding yeast. EB1 proteins are conserved microtubule tip-trackers, forming a platform for a range of proteins, which they recruit to the microtubule tip [4,5], including members of the kinesin-14 family [6].

In their experiments, Molodtsov *et al.* nucleated radially growing microtubules from *Tetrahymena* pellicles serving as microtubule-organizing centers. They observed that when the Cik1–Kar3 kinesin, hitchhiking on the growing microtubule tip using EB1, encounters a pre-existing microtubule lattice it crosslinks to the other microtubule through its motor domain and thus

guides the tip of the growing microtubule along the pre-existing one. The movement is driven by microtubule polymerization, while tip-associated kinesin-14 is solely enabling the guidance (Figure 1).

To achieve this guidance, kinesin-14 has to move toward the plus-tip of the microtubule, as all the microtubules emanating from the microtubule-organizing center grow with their plus-tips outwards. This means that the minus-end-directed kinesin-14 has to move backwards. Some molecular motors are known to be able to backstep under several piconewton-high external loads opposing their stepping [7]. In their beautiful quantitative *in vitro* measurement using optical tweezers, Molodtsov *et al.* show that Cik1–Kar3 kinesin-14 is a rather weak motor, which readily backsteps already at sub-piconewton opposing loads. Single growing microtubule tips, generating forces of several piconewtons [8], can thus easily reverse the movement of multiple Cik1–Kar3 motors, which might be located simultaneously at the tip and work in parallel. This tug-of-war between the polymerizing microtubule tip and an ensemble of kinesin-14 motors is nicely visualized in one of the experiments by Molodtsov *et al.*, in which they show that the speed of the microtubule polymerization decreases with the increasing amount of kinesin-14 motors at the microtubule tip, indicating that guidance comes at the cost of reduced microtubule polymerization velocity. This decrease in microtubule growth is dependent on the motor activity, as it is seen only in the presence of ATP; however, microtubule guiding does not depend on ATP, as the microtubules are guided by Cik1–Kar3 in the presence of both ATP or ADP. The reduced microtubule polymerization velocity indicates that cells may not always use the most cost-efficient way to fulfill a task, but instead adapt to function with the material at hand — in this example, instead of a diffusible crosslinker, which would likely be sufficient to drive the guiding, a motor with a propensity for backstepping is employed.

The consequence of the guided polymerization is the formation of parallel bundles emanating from the microtubule-organizing center. Molodtsov *et al.* show that Cik1–Kar3-dependent parallel bundling is important for building a

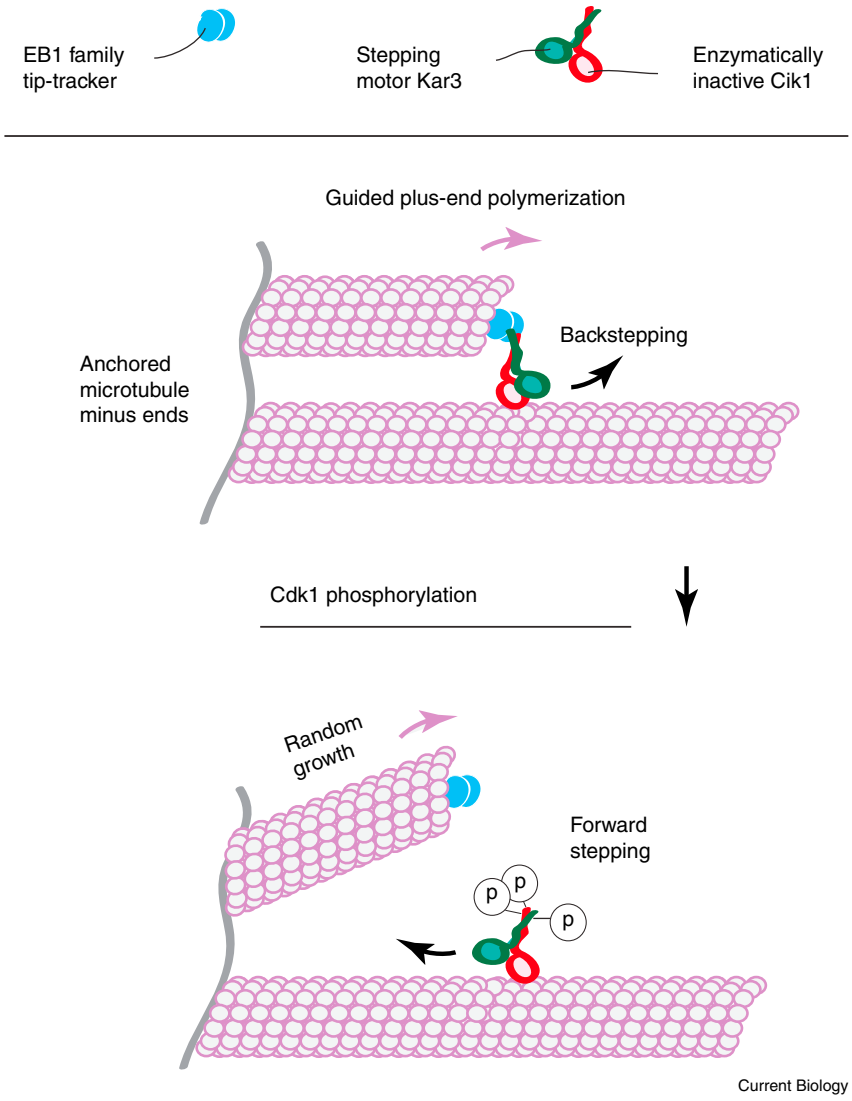

Figure 1. Backstepping kinesin-14 guides growing microtubules along pre-existing ones.
Plus-end polymerization of growing microtubules is guided along pre-existing microtubules by EB1 and Cik1–Kar3-dependent crosslinking, during which the heterodimeric kinesin-14 Cik1–Kar3, pushed backwards by the growing microtubule tip, processively backsteps. Upon Cdk1-dependent phosphorylation of Cik1–Kar3, the kinesin-14 is decoupled from EB1 at the tip of the growing microtubule. While Cik1–Kar3 now processively moves forward to the microtubule minus-end, the decoupled nascent microtubule grows in a random direction and is no longer bundled in parallel with the pre-existing microtubule.

parallel microtubule bundle at the budding yeast shmoo tip. Interestingly, (de)phosphorylation of the kinesin-14 acts like a switch for the sorting mechanism as only dephosphorylated motors can bind to EB1 and thus hitchhike on the growing microtubule tip. The authors beautifully show *in vitro* that Cik1–Kar3, if decoupled from EB1 on the polymerizing microtubule tip by phosphorylation, processively walks to the microtubule minus-end, while the uncoupled microtubule grows off in a random direction (Figure 1). Local phosphorylation of the motors could thus give rise to asters coexisting with bundles of parallel microtubules in other parts of the cell (for example a shmoo tip), explaining the microtubule arrangement in the mating budding yeast [9].

What are the underlying reaction kinetics of the observed kinesin-14-mediated microtubule guiding? Unlike kinesin-14 motors from other organisms, budding yeast Cik1–Kar3 is a processive molecular motor, which means that it can take several steps along the microtubule

lattice — potentially also backwards — before unbinding [10]. Nevertheless, in their paper, Molodtsov *et al.* show that the guiding mechanism they describe also works for other, non-processive kinesin-14 motors, for example *Drosophila* Ncd and human HSET, which are thought to interact with the microtubule only very briefly, taking only one step before detachment. Similarly, it has been shown that EB1 family proteins turn over very rapidly on the microtubule tip [11]. Therefore, microtubule guiding may not rely only on motor back-stepping but also on accelerated motor rebinding: as the kinesin-14–EB1 complex forms a crosslink between the two microtubule lattices, it is conceivable that, when the complex unbinds from one of the lattice binding sites, it is held in close vicinity to this site by its interaction with the other binding site. This could facilitate its fast rebinding and prolong the effective dwell time of the crosslinker complex between the two microtubules. It is thus tempting to speculate that the crosslinking activity actually changes the kinetics of the two interaction sites and stabilizes the complex between the two microtubules. Future studies on the kinetics of kinesin-14-mediated microtubule guiding should yield interesting results and help to understand the actions of this intriguing molecular motor.

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Evolutionary Genetics: Smells like a Pseudo-pseudogene

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A new study reports the presence of a chemosensory pseudogene in *Drosophila sechellia*, which in spite of carrying a premature stop-codon nevertheless encodes a fully functional and full-length protein. Such 'pseudo-pseudogenes' might well be a widespread phenomenon.

In 1977, George Brownlee and colleagues made an intriguing discovery in the genome of the African clawed frog *Xenopus laevis*, namely a truncated copy of the 5S rRNA gene [1]. This DNA fragment evidently did not generate a functional product and hence they termed their new discovery a 'pseudogene'. With regards to its presence in the frog genome, Brownlee and co-workers felt "*forced to the conclusion that the most probable explanation for the existence of the pseudogene is that it is a relic of evolution*". Today, we know that pseudogenes are frequent and, as Brownlee and colleagues rightly concluded, relics in the genome, present as leftovers from the birth-and-death process of molecular evolution. The human genome contains ~14,000 identifiable pseudogenes [2], a figure not that different from the estimated total number of functional genes (~19–20,000) [3]. Analysis of the presence and absence of pseudogenes has also revealed important insights into a wide range of topics, including, for example, chemosensory evolution [4] and adaptations towards aquatic life [5]. But, not so fast! A new study by Richard