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Cell Polarity: A New Mod(e) of Anchoring

Dispatch

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Microtubules play a central role in the establishment of cell polarity by directing the transport of polarity determinants to their site of action. Recent work has revealed a novel membrane-anchoring mechanism which complements the microtubule transport of the fission yeast polarity determinant tea1p to ensure its retention at the cell tip.

Cell polarity is a fundamental property of eukaryotic cells, important both for their function and, in the case of metazoans, for development of the organism as a whole. To establish cell polarity, determinants that specify cell fate or cell shape are transported to specific locations in the cell along microtubule tracks. In *Drosophila* oocytes, for example, germline determinants accumulate at the posterior of the cell by a mechanism that involves their transport towards microtubule plus-ends [1]. In neurons, the polarised microtubule cytoskeleton guides the kinesin- and dynein-mediated movement of proteins and RNA molecules to their respective axonal and dendritic compartments [2]. The microtubule cytoskeleton is also important for the migration of mammalian fibroblasts, and appears to contribute to the transport of secretory vesicles and the regulation of the actin cytoskeleton and of focal adhesions [3]. Although directed transport of determinants is important in all these cells, in most cases, it not known whether microtubule transport is sufficient for localisation, or whether mechanisms are also required to anchor the determinants in specific locations.

The fission yeast *Schizosaccharomyces pombe* is an excellent species in which to study three-dimensional organisation, as the precise localisation of growth sites to opposite ends of the cell is necessary to maintain its characteristic rod-shape. To grow as a rod, a fission yeast cell must position polarity factors and actin in a spatially accurate way at the cell tips. A dynamic system of microtubules appears to be responsible for keeping these cells growing straight [4]. A fission yeast cell contains three to five bundles of microtubules, which are nucleated from interphase organising centres located around the centrally placed nucleus [5,6]. Each of the microtubule plus ends grows towards the cell tip, touches it for 1–2 minutes and then shrinks back to the nucleus. Regulation of such microtubule 'catastrophes' ensures that microtubules generally only shrink upon contact with the cell tips [7].

These dynamic microtubules play a central role in cell morphogenesis, in part by mediating the localisation of the kelch-repeat protein tea1p to cell tips [8]. Tea1p is necessary for the maintenance of diametrically opposed growth sites: mutations in *tea1* lead to misplacement of the site of growth to an ectopic central location and the consequent formation of T-shaped cells [8]. Tea1p is located on the plus ends of microtubules and in additional dots concentrated at the cell tips. Live observation using a tea1p fusion with the green fluorescent protein (GFP) has shown that tea1p-containing particles travel on the plus ends of growing microtubules towards cell ends; when the microtubule depolymerises, tea1p appears to be left behind at the cortex [9] (F.C., unpublished observations). Tea1p localisation on microtubule plus ends also depends on tea2p, a kinesin-like protein, and tip1p, the *S. pombe* homologue of the microtubule plus-end-binding protein CLIP170, highlighting the essential role of microtubule plus-end-directed transport in this process [7,10].

Recent work by Snaith and Sawin [11] has now shown that, once tea1p is delivered to the cell tip by the microtubule, it needs to be docked by a novel membrane-anchoring mechanism. In a genetic screen for mutants exhibiting aberrant morphology, the authors identified a novel gene *morphology defective 5* (*mod 5*): *mod5*Δ mutant cells show a low frequency of T-shaped cells, a phenotype similar to, though weaker than, that of *tea1*Δ mutants. Although tea1p is still localised properly to the microtubule plus ends, the cell tip localisation of tea1p is dramatically reduced, and the residual tea1p dots positioned at cell tips are ones still on the microtubule plus-ends. Time-lapse microscopy suggests that, in a *mod5*Δ mutant, tea1p particles either disassemble or move back with the shrinking microtubule. When all the microtubules are depolymerised, tea1p is absent from the cell ends in mod5Δ cells. This phenotype suggests that *mod5*Δ mutants are defective in docking or stabilising tea1p at the cell tip.

Mod5p has characteristics expected of a membrane anchor: it is a novel protein with a prenylation site (CaaX motif) responsible for its plasma membrane localisation. Mod5p is concentrated at the membrane in caps at the cell tips where tea1p is deposited. As sterol-rich membrane domains — indicative of lipid rafts — are also concentrated at cell tips, lipid rafts in the plasma membrane may contribute to the polarised distribution of the prenylated mod5p [12]. The mod5p amino-acid sequence, however, does not give any strong hints as to how it may function to anchor tea1p. Whether mod5p directly binds to tea1p or acts through intermediaries is not yet known. Although actin networks have been proposed to anchor organelles at the cortex [13], mod5p probably does not act through actin, as the docking of tea1p has previously been shown to be actin independent [8].

These results suggest a two-step model for the stable positioning of tea1p at cell tips (Figure 1). First tea1p particles are transported towards the cell tips

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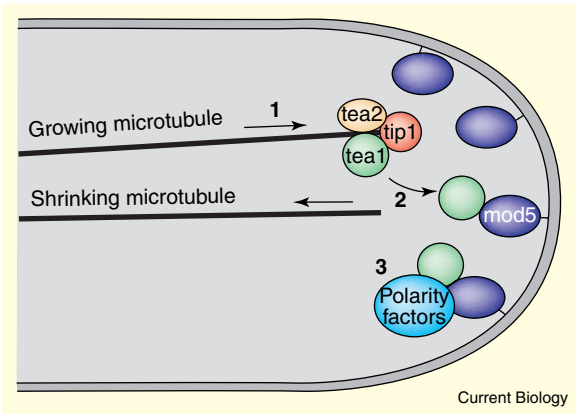

Figure 1. A model for the localisation of tea1p to cell tips in fission yeast.

(1) Tea1p is transported to cell tips on the plus-ends of growing microtubules, in a tip1p- and tea2p-dependent manner. (2) Coincident with microtubule shrinkage, tea1p is released from microtubules and stabilised at the plasma membrane in a mod5p-dependent manner. Mod5p is anchored in the plasma membrane by virtue of its prenylation. (3) Tea1p functions to regulate cell polarity by associating with other polarity factors.

on growing microtubule plus-ends in a tip1- and tea2-dependent manner. When the microtubule shrinks back, tea1p is released from the microtubule and anchored to the plasma membrane in a mod5p-dependent manner.

This model raises the question of how tea1p is released from the microtubules and transferred to its docking site. In *mod5*Δ mutants, tea1p dots are observed to exhibit bidirectional movements, implying that, in the absence of its cortical anchor, tea1p remains bound to at least some depolymerising microtubules. This observation suggests that docking may help to release tea1p from the microtubule. Microtubule shrinkage may be sufficient to lower the affinity of tea1p for the microtubule and favour binding to its cortical partner. Alternatively, tea1p may be actively removed from the microtubule.

One possibility is that tea1p may be post-translationally modified by an enzyme located at the cell tip, inducing a decrease in its affinity to the microtubule and an increase in its affinity to the cortical anchor. Interestingly, tea1p has recently been shown to be phosphorylated by the kinase shk1p, a member of the Pak family of serine/threonine kinases which is located at cell tips [14,15]. In support of the idea that this phosphorylation event is necessary for tea1p function, *shk1* mutant cells exhibit patterns of growth very similar to that of *tea1*Δ cells [14]. But the possibility that phosphorylation by shk1p plays a role in the docking of tea1 still remains to be examined.

In this hand off from the microtubule to the cortex, tea1p may be transferred from one set of binding partners to another. Indeed, tea1p fractionates in three different complexes of large molecular weight [16]. Interestingly, these complexes appear to contain different proteins; one tea1p complex contains tip1p, while the others contain polarity factors such as bud6p and the formin for3p [16] (B. Feierbach and F.C., unpublished observation). It will be interesting to determine the possible effects of mod5p and shk1p on these complexes

Although the two-step model described above is attractive for its simplicity, a number of results suggest that the relationship between tea1p and mod5p may be more complex. Not only is mod5p necessary to localise tea1p at cell tips, tea1p is needed for mod5p localisation [11]. Indeed, in *tea1*Δ mutants, mod5p is now evenly distributed all around the cortex (although *tea1*Δ cells still grow in a polarised manner at one tip). Tea1p and mod5p may thus be part of a positive feedback mechanism that promotes each other's localisation. This mechanism may ensure a precise, but dynamic placement of growth sites, allowing for rapid disassembly of a site of growth, for instance when a cell needs to orient towards its mating partner [17].

The discovery of mod5p and its function in the maintenance of tea1p localisation highlights the point that transport mechanisms along cytoskeletal elements may not be sufficient to ensure accumulation of determinants at their site of action. Anchoring of determinants to distinct membrane domains also plays an important role in cell polarisation and closely cooperates with transport mechanisms to promote their spatial restriction. Future research should tell us whether this principle extends to other polarised cell types.

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