

- plasticity in neocortical microcircuits. *Prog. Brain Res.* 169, 211–223.
14. Alvarez, V.A., and Sabatini, B.L. (2007). Anatomical and physiological plasticity of dendritic spines. *Annu. Rev. Neurosci.* 30, 79–97.
  15. Stettler, D.D., Yamahachi, H., Li, W., Denk, W., and Gilbert, C.D. (2006). Axons and synaptic boutons are highly dynamic in adult visual cortex. *Neuron* 49, 877–887.
  16. Gogolla, N., Galimberti, I., and Caroni, P. (2007). Structural plasticity of axon terminals in the adult. *Curr. Opin. Neurobiol.* 17, 516–524.
  17. Lee, W.C., Huang, H., Feng, G., Sanes, J.R., Brown, E.N., So, P.T., and Nedivi, E. (2006). Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex. *PLoS Biol.* 4, e29.

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## Cell Division: Experiments and Modelling Unite to Resolve the Middle

**How does a cell find its middle? New experiments confirm that activating stable microtubules together with inhibitory dynamic microtubules are key ingredients. Quantitative modelling has now further scrutinised these ideas, yielding fresh insights into furrow positioning.**

**Martin Howard**

The question of how a cell specifies the position of its cytokinetic furrow, and can thereby divide into two appropriately-sized daughter cells, has long fascinated cell biologists. The factors that influence furrow positioning have remained frustratingly obscure, however, despite decades of experimentation. One central question is whether it is a positive signal to the cell equator or negative signals to everywhere else on the cortex, or some combination of both, that leads to specification of the furrow position [1]. Two new experimental studies [2,3] and one computational modelling paper [4] have now significantly clarified the issue.

One possible mechanism through which the furrow could be positioned is via microtubules, which could deliver regulatory signals to the cell cortex. A critical positive regulator of cytokinesis is the small GTPase Rho, whose active form is known to localise to the equatorial cortex before furrow ingression, thereby promoting the recruitment of both actin and myosin. Rho is in turn regulated by a Rho guanine exchange factor (RhoGEF) and by centralspindlin, a complex of the microtubule-binding motor protein MKLP1 and a Rho GTPase-activating protein (RhoGAP), all of which also concentrate at furrows. Here, RhoGAP binds to RhoGEF, which in turn generates active Rho. Most microtubules are highly dynamic, undergoing successive cycles of rescue and catastrophe. However, previous work had

uncovered the existence of a subset of unusually stable microtubules that tended to point towards the equatorial cortex, precisely the region of furrow formation [5]. Using centralspindlin, these stable microtubules could potentially deliver the activator RhoGEF directly to the site of furrow initiation.

The vital importance of stable versus dynamic microtubules has now been further underlined in the new studies through the use of precisely timed treatment with the microtubule polymerization inhibitor nocodazole. Murthy and Wadsworth [2] examined the effects of nocodazole on fluorescently labelled Rho and actin in kidney epithelial LLC-Pk1 cells, whereas Foe and von Dassow [3] investigated its effects on myosin activation using antibodies against the serine 19-phosphorylated form of myosin regulatory light chain (pRLC), a stimulatory phosphorylation, in fixed sea urchin zygotes.

In sea urchin zygotes without drug treatment, levels of pRLC were initially uniform throughout the cortex during prophase and metaphase, before being globally reduced at the start of anaphase, after which pRLC levels increased but only at the equatorial cortex associated with the furrow. Prior to anaphase, essentially all non-kinetochore microtubules were dynamically unstable and were therefore depolymerised by nocodazole treatment. During early anaphase, astral microtubules were observed to extend towards the cortex. Nocodazole treatment at this time again caused depolymerisation of the

predominantly dynamic microtubules, and this correlated with inhibition of furrowing. However, later during anaphase, treatment with nocodazole failed to prevent abscission. In fact, successful division was found to correlate with a stable subset of nocodazole-resistant microtubules that pointed to the equatorial cortex. In this case, compared to untreated cells, pRLC concentrations at the cortex and furrow width were both increased. More generally, in cells that completed division, high levels of pRLC during anaphase were always associated with stable microtubule tips at the equatorial cortex, whereas lower pRLC levels elsewhere correlated with nearby dynamic microtubules. For the case of LLC-Pk1 cells, similar results were found, with the additional interesting finding that fluorescently labelled actin exhibited unusual travelling wave-like behaviour in nocodazole-treated cells.

Previous work had uncovered the presence of two specifying signals from the mitotic apparatus that regulate furrow positioning [6]. Here, stable microtubules appear to be an activating factor for furrow formation, as found previously [5]. Furthermore, the correlation between low pRLC levels and proximity to dynamic microtubules, and the increase in furrow width and pRLC intensity in nocodazole-treated cells, indicate that dynamic microtubules are an important inhibitory component of the furrow-positioning system. This finding confirms and extends previous work that implicated astral microtubules in inhibiting furrowing [7].

How can these two conflicting roles for microtubules be resolved? Both Murthy and Wadsworth [2], and Odell and Foe [4] suggest a similar resolution. Stable equatorial microtubules are able to deliver high enough concentrations of positive regulators to reliably induce furrowing. However, the dynamic nature of other astral microtubules prevents the delivery of these positive

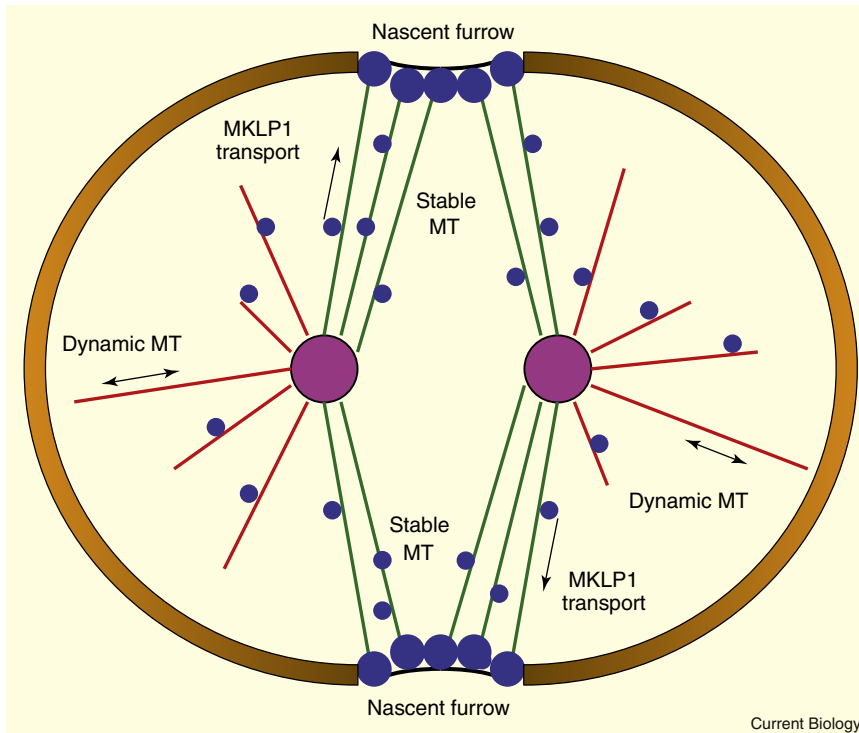


Figure 1. Schematic figure indicating how stable equatorial microtubules promote, but dynamic astral microtubules inhibit, furrow formation.

Centrosomes are shown in pink, stable microtubules (MT) are green, dynamic microtubules are red, small blue circles are individual MKLP1 molecules walking along microtubules, large blue circles are MKLP1 accumulations at stable microtubule tips. A hypothetical polar inhibitor gradient on the cortex is in orange, with bright orange corresponding to high concentrations of the inhibitor. Components of the central spindle are omitted for clarity.

regulators of furrow formation to non-equatorial regions of the cortex. In effect, dynamic microtubules undergo such a high rate of catastrophe that they only transiently reach the cortex and, as such, are unable to transport furrow-activating factors at high enough concentrations to the cortex to induce furrowing. Such a hypothesis, illustrated in Figure 1, is undeniably attractive. However appealing such a cartoon model may be, the question is: will it actually work? In such a complex system as furrow positioning, with competing spatiotemporal interactions, intuition can be unreliable. Instead, a more systematic way to proceed is to develop a mathematical model of the system. In that way the dynamics can be quantitatively dissected in order to uncover the necessary ingredients for precise furrow positioning. Setting up and testing such a model was the goal of the modelling paper by Odell and Foe [4].

Their model incorporates stochastically growing microtubules

that effectively undergo catastrophe after hitting the cell cortex. After a catastrophe, such a microtubule then shrinks until stochastic rescue or else depolymerises away completely. In the latter case, the depolymerised microtubule is eventually replaced by another microtubule growing from the centrosome in a random direction. A subset of microtubules pointing towards the equatorial cortex is assumed to be stable, meaning that for these microtubules no shrinkage occurs during catastrophes. The dynamics of MKLP1 are then simulated on top of this system by allowing MKLP1 to diffuse in the cytoplasm and also bind/unbind to microtubule tracks along which it can walk at a fixed speed. At microtubule tips, MKLP1 was initially assumed to be able to simply 'walk' off.

Importantly, such a model did not give rise to a sufficiently robust build-up of MKLP1 (and hence of active Rho) at the furrow. In essence, the random diffusion of MKLP1 was sufficient to undo the microtubule-dependent accumulation

of MKLP1, thereby leading to only very modest enrichment at the furrow, which is unlikely to be a sufficiently reliable signal. However, if MKLP1 was assumed to stall at microtubule tips (i.e. it was unable to walk off, but could only be removed by normal unbinding) then the whole situation changed. Now very substantial accumulations of MKLP1 could be found at the equatorial cortex, likely sufficient to induce robust furrowing. Moreover, such tip accumulations have previously been seen in experiments [8], adding further support to the hypothesis. The model was also able to deal with the nocadazole experiments: eliminating all but stable microtubules caused levels of MKLP1 at the equatorial cortex to increase, as the MKLP1 previously sequestered by dynamic microtubules was released.

Clearly, experiment and modelling now agree on the importance of stable, activating microtubules at the equatorial cortex together with inhibitory, dynamic microtubules that only transiently reach the cortex. However, a number of mysteries remain. These include the behaviour of pRLC in cells treated with colchicine to reduce the size of the mitotic apparatus [3]. In this case, pRLC was still concentrated in a diffuse equatorial band, even though no microtubules contacted the equator. It is currently unknown whether the model can handle this situation. Moreover, as pointed out by the authors themselves [4], what makes the microtubules contacting the equatorial cortex privileged in their stability? At the moment such a property is an input into the model, whereas in reality the cell must find a way to generate such patterning spontaneously.

Clearly, there must be other mechanisms regulating furrow positioning. Another motif that recurs in cell-division positioning in more primitive cells is that of a polar repressing gradient. In this context, a concentration gradient high at the poles and low at the equatorial cortex could modulate microtubule stability. Concentration gradients are generated in bacterial cell division [9] and also in division in fission yeast [10]. Moreover, similar chemical gradients that alter microtubule stability are believed to occur in the context of spindle assembly [11]. However, in order for such gradients to generate reliable

positioning, the peaks of the gradient must identify opposite cell poles, so that the concentration minimum is appropriately at mid-cell. In rod-shaped bacteria or fission yeast this identification is relatively straightforward but could be a more challenging proposition in the more complex cell shapes seen in higher eukaryotes. Perhaps the orientation of the kinetochore microtubules can provide a cue. In any case, such a gradient, if it exists, could be used as a redundant mechanism to position the furrow, for example, in colchicine-treated cells where the primary microtubule-based mechanism may be less effective.

A final important issue raised by the modelling is more philosophical in nature, namely how should one go about constructing a mathematical model of an intrinsically complex biological system. The approach adopted by Odell and Foe is a three-dimensional agent-based model, where hundreds of thousands of differential equations are solved so that the paths of individual cytoskeletal agents, such as molecules of MKLP1, can be followed. Such a model produces a detailed picture of the cell at a microlevel, but at the cost of building a model whose complexity is perhaps not that much

simpler than the cell itself. Of course, the model can be more easily manipulated and its fundamental elements controlled, but it is still undeniably a complex creation. An alternative modelling philosophy is to try to construct the simplest set of equations possible and see how far a more minimalist approach can lead, often by losing track of individual molecules and modelling at a mesoscale. Such an approach has recently been taken, for example, to examine the polarisation of the one-cell *Caenorhabditis elegans* embryo [12]. Clearly, both approaches have their merits, and ultimately both will probably be needed. Regardless of the detailed methodology, however, it is clear that mathematical modelling of cell-division positioning is one area where modelling has, for once, lived up to the hype.

#### References

1. Burgess, D.R., and Chang, F. (2005). Site selection for the cleavage furrow at cytokinesis. *Trends Cell Biol.* 15, 156–162.
2. Murthy, K., and Wadsworth, P. (2008). Dual role for microtubules in regulating cortical contractility during cytokinesis. *J. Cell Sci.* 121, 2350–2359.
3. Foe, V.E., and von Dassow, G. (2008). Stable and dynamic microtubules coordinately shape the myosin activation zone during cytokinetic furrow formation. *J. Cell Biol.* 183, 457–470.
4. Odell, G.M., and Foe, V.E. (2008). An agent-based model contrasts opposite effects of dynamic and stable microtubules on cleavage furrow positioning. *J. Cell Biol.* 183, 471–483.
5. Canman, J.C., Cameron, L.A., Maddox, P.S., Straight, A., Tirnauer, J.S., Mitchison, T.J., Fang, G., Kapoor, T.M., and Salmon, E.D. (2003). Determining the position of the cell division plane. *Nature* 424, 1074–1078.
6. Bringmann, H., and Hyman, A.A. (2005). A cytokinesis furrow is positioned by two consecutive signals. *Nature* 436, 731–734.
7. Werner, M., Munro, E., and Glotzer, M. (2007). Astral signals spatially bias cortical myosin recruitment to break symmetry and promote cytokinesis. *Curr. Biol.* 17, 1286–1297.
8. Nishimura, Y., and Yonemura, S. (2006). Centralspindlin regulates ECT2 and RhoA accumulation at the equatorial cortex during cytokinesis. *J. Cell Sci.* 119, 104–114.
9. Howard, M., and Kruse, K. (2005). Cellular organization by self-organization: mechanisms and models for Min protein dynamics. *J. Cell Biol.* 168, 533–536.
10. Padte, N.N., Martin, S.G., Howard, M., and Chang, F. (2006). The cell-end factor Pom1p inhibits Mid1p in specification of the cell division plane in fission yeast. *Curr. Biol.* 16, 2480–2487.
11. Bastiaens, P., Caudron, M., Niethammer, P., and Karsenti, E. (2006). Gradients in the self-organization of the mitotic spindle. *Trends Cell Biol.* 16, 125–134.
12. Tostevin, F., and Howard, M. (2008). Modeling the establishment of PAR protein polarity in the one-cell *C. elegans* embryo. *Biophys. J.* 95, 4512–4522.

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## Mammalian Vision: Rods Are a Bargain

To maintain resting potentials in darkness, rod and cone photoreceptors incur a significant energy cost. But in brighter light, rods become energetically ‘cheaper’ than cones, which might explain the evolution of the vertebrate duplex retina.

### Eric J. Warrant

After pondering the absence of eyes in animals living in the pitch dark, such as those living in deep caves, Charles Darwin [1] wrote: “As it is difficult to imagine that the eyes, though useless, could be in any way injurious to animals living in darkness, their loss may be attributed to disuse”. For Darwin, the absence of any selection pressure to retain a sensory structure that provides no information to its owner — no fitness benefit — should ultimately lead to

that structure’s demise. What Darwin could not have known in 1859 was that quite aside from the lack of benefits, retention of a useless sensory structure would also incur a significant cost, the currency of which is energy [2]. It would be more than a century before we began to understand the implications of this cost in natural selection, and to understand the inevitable ‘cost-benefit’ analysis that must occur during the evolution of the senses [3].

Animal photoreceptors are an excellent case in point. Even in total darkness, as would be experienced by a cave-dwelling animal, photoreceptors consume a considerable amount of energy — in the form of ATP molecules — solely to maintain their resting potentials in readiness for a light stimulus [4–6]. The major part of this cost goes on fuelling the pumps that transport Na<sup>+</sup> and K<sup>+</sup> ions across the cell membrane, thereby keeping the resting potential at steady state. In all photoreceptors studied to date, this cost only increases as light levels rise (Figure 1B), because of the inevitable extra costs associated with signalling [6]. But now, in a landmark study published recently in *Current Biology*, Okawa *et al.* [7] have shown that, in mammalian rod photoreceptors, this is not the case. Instead of consuming more energy as light levels rise, rods