CYTOSKELETON

A speckled spindle

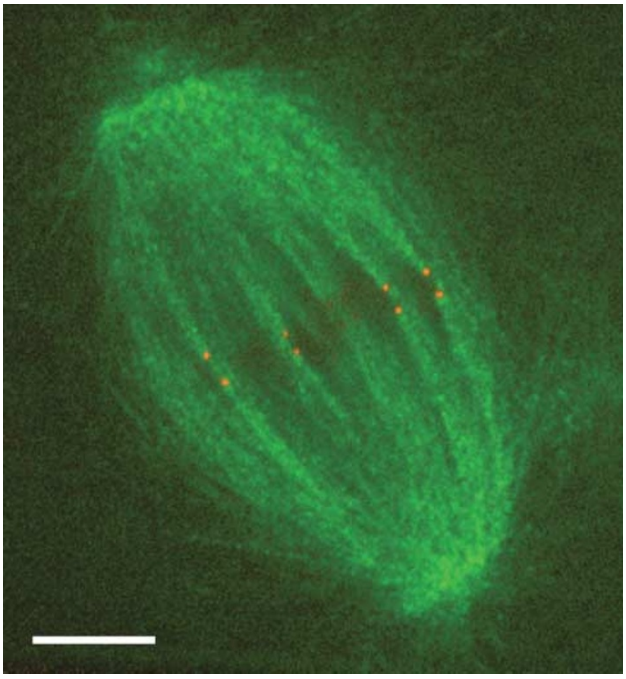

Image courtesy of Paul Maddox, University of North Carolina, Chapel Hill, USA. Scale bar, 5 μm.

Behind the scenes, much of what appears static in the cell is actually the steady state of a dynamic system. The cytoskeleton is a key example: cytoskeletal polymers such as actin filaments and microtubules regularly undergo polymerization and depolymerization, which is often not visible if the overall length of the polymer remains unchanged. To really get a handle on these polymer 'fluxes', you need to ask what is going on with individual polymer subunits — a challenge that has been met by fluorescent-speckle microscopy. In the August issue of *The Journal of Cell Biology*, Maddox *et al.* apply this technology to the study of kinetochore microtubules, and from their observations they provide new insights into the microtubule dynamics that drive spindle mechanics.

During mitosis, kinetochore microtubules produce force that eventually pulls chromosomes towards the spindle poles. Numerous studies have looked at the contributions made during this process by kinetochore microtubule poleward flux versus the force generated at the kinetochore itself. However, the results have varied depending on the system examined and were sometimes limited by the inability to distinguish kinetochore microtubules from other spindle microtubules.

Maddox *et al.* set out to distinguish between these two models by using fluorescent-speckle microscopy on spindles in *Xenopus* egg extracts. The premise of this method is that low levels of fluorescent-tubulin subunits will co-polymerize with non-labelled subunits into a microtubule polymer and so provide reference marks that allow the movements of individual subunits within the polymer to be followed (shown in green in the figure; kinetochores are selectively labelled red). Taking this approach, Maddox *et al.* asked whether kinetochore microtubules do indeed flux poleward.

They saw that during metaphase, kinetochore microtubules do flux, and thereby create tension at the kinetochore. After anaphase entry, these microtubules switch to depolymerization at the kinetochore. This, combined with the poleward flux, seems

IMAGING TECHNIQUE

Mind-reader

The ability to image the molecular structure of the brain has provided us with insights into its normal and pathological functions. However, the technologies that are available to image neuroanatomy are generally slow. Automated, high-throughput methods similar to those applied to the field of proteomics would therefore be invaluable, and such a method might now be available. In *Neuron*, Kleinfeld and colleagues present a technique — all-optical histology — for the automated, three-dimensional (3D) histological analysis of brain tissue.

Recent work indicated that ultra-short amplified laser pulses could be used to section or remove layers of tissue. The potential to use these lasers as a histological tool fits well with the fact that similar nonamplified laser pulses are used in the imaging technique two-photon laser-scanning microscopy (TPLSM). TPLSM can be used to image fluorescent molecules to depths of hundreds of microns from the brain surface.

Repeated rounds of ablation and imaging could therefore be used to obtain diffraction-limited volumetric data that can be used to reconstruct fluorescently labelled tissue in 3D. Advantages of this method include the fact that it can be carried out on unfrozen samples and that the physical location of the sample can be maintained, which means that this process could be automated. And, it seems that there are few disadvantages.

Kleinfeld and co-workers showed that 1–10 J laser pulses could be used to make cuts that could ablate tissue with micron precision, and that the roughness of the optically ablated surface is comparable to that of frozen or unfrozen surfaces that have been cut using a knife. The roughness of the block face is also well within the imaging depth of TPLSM, which makes the ablation and imaging methods compatible. Furthermore, they tested this method on fragile, unfrozen embryonic mouse brain and found that both the gross and fine structure appeared normal after large-scale laser tissue removal.

Next, the authors showed that, after ablation, normal tissue properties are retained in the adjacent, unablated regions. For example, they applied fluorescent dyes to show that the physical integrity of the cell surface and organelles had been preserved. Furthermore, they showed the preservation of antigenic response, and the retention of fluorescence in tissue from transgenic animals expressing fluorescent proteins.

In the final part of this work, Kleinfeld and colleagues used all-optical histology to generate 3D reconstructions of labelled tissue. In the first example, they imaged, with micron resolution, the fixed neocortex of transgenic mice in which certain neurons were selectively expressing yellow fluorescent protein. In the second example, they imaged, again with micron resolution, the microvasculature in a block of fixed neocortex from transgenic mice expressing cyan fluorescent protein. This work has therefore shown that the use of laser pulses to ablate and then image fixed and fresh tissue could provide us with a powerful method for the automated, high-throughput, 3D histological analysis of brain tissue.

Rachel Smallridge

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WEB SITE

David Kleinfeld's laboratory:
http://www-physics.ucsd.edu/neurophysics/

to drive chromosomes poleward. Variable switching between persistent polymerization and depolymerization was often observed in anaphase. The authors propose that switching to polymerization provides a 'clutch' mechanism that prevents microtubule detachment following strong force.

From these studies, new implications for spindle dynamics emerge. In particular, the authors propose that all kinetochores are fundamentally bistable, and that different behaviours such as chromosome oscillations depend on different flux rates within the microtubule polymer. This work therefore provides an illustrative example of the power of fluorescent-speckle microscopy and the key tool it has become for analysing cytoskeletal function in live cells.

Alison Schuldt, Associate Editor,
Nature Cell Biology

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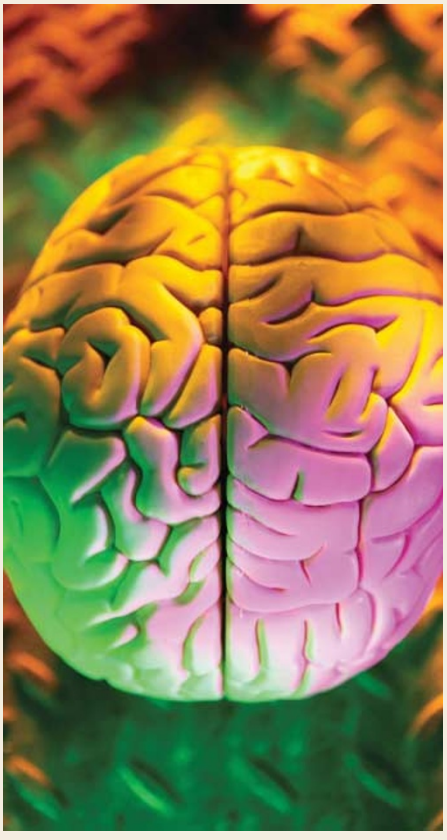

SIGNAL TRANSDUCTION

Trigger-happy trio

Upstream of the small GTPase Rac1, a key cytoskeletal regulator, lie Elmo (engulfment and cell motility protein) and Dock180. A signalling cascade from another small GTPase, RhoG, activates Rac1. A report by Katoh and Negishi in *Nature* now links these components, and shows that RhoG, through a ternary complex with Elmo and Dock180, triggers activation of Rac1.

Like Rac1 and Cdc42, RhoG mediates cellular morphological processes. Unlike Rac1 and Cdc42, however, it doesn't bind known Rac1 and Cdc42 effectors, so the authors used constitutively active RhoG (V12RhoG) in a yeast two-hybrid screen to look for potential binding partners. One positive clone encoded Elmo 2, an upstream regulator of Rac1. Using pull-down assays, Katoh and Negishi saw that Elmo interacted only with GTP-bound, but not GDP-bound, RhoG, and that the amino terminus (residues 1–362) of Elmo was sufficient for the interaction. Co-transfected V12RhoG and Elmo also interacted in mammalian cells. Elmo did not interact with V12Rac1, V12Cdc42 or V14RhoA — and dominant-negative RhoG (N17RhoG) and V12RhoG with a phenylalanine→alanine substitution (F37A) in the effector region (which can't induce morphological changes) also failed to interact with Elmo.

Elmo is already known to interact — through its carboxyl terminus — with Dock180, raising the possibility that a RhoG–Elmo–Dock180 complex could form. RhoG and Dock180 co-immunoprecipitated in mammalian cells, but only when Elmo was expressed. If the carboxyl terminus of Elmo, which binds Dock180, was deleted (Elmo-T618), RhoG and Dock180 did not interact.

Dock180 and Elmo localize mainly in the cytoplasm, but Dock180 recruitment to the plasma membrane might be important in its cytoskeletal role. So, does RhoG have a role in this re-localization? Co-expression of V12RhoG did indeed cause the co-localization of Dock180 and Elmo with V12RhoG at the plasma membrane.

Not only does RhoG influence Dock180's localization, but V12RhoG also enhances the Dock180- and Elmo-mediated activation of Rac1. Elmo-T618, which does not interact with Dock180, blocked the V12RhoG-induced augmentation of Dock180 activity to Rac1. This indicates that RhoG, through Elmo, promotes Dock180-induced Rac1 activation. As Elmo-T618 inhibited the ability of V12RhoG to induce membrane ruffling, the Elmo–Dock180 interaction seems to be required for this cytoskeletal change. Similarly, a mutant form of Dock180 (Dock180-ISP→AAA) that binds Elmo, but that doesn't activate Rac1, inhibited membrane ruffling.

Dock180 functions downstream of integrin signalling, so the authors investigated whether RhoG and Elmo were involved. N17RhoG, Elmo-T618 or Dock180-ISP→AAA all suppressed the spreading of HeLa cells on fibronectin. Katoh and Negishi also saw that Rac1 was activated when 293T cells were plated on fibronectin, and that this was blocked by Elmo-T618 or Dock180-ISP→AAA, pointing to an essential role for RhoG–Elmo–Dock180 signalling in integrin-mediated Rac1 activation and cell spreading.

Finally, RhoG is known to mediate neurite outgrowth, and, perhaps not surprisingly, Elmo and Dock180 are now implicated in nerve-growth-factor-induced neurite outgrowth downstream of RhoG — indeed, Elmo-T618 or Dock180-ISP→AAA blocked this.

So RhoG, Elmo and Dock180 are important upstream regulators of Rac1. And because previous results indicate that RhoG is a target of the guanine-nucleotide exchange factors Trio and Kalirin, the authors propose that the newly found pathway — RhoG–Elmo–Dock180 — that regulates Rac1 activity during neurite outgrowth, extends upwards to Trio/Kalirin.

Katrin Bussell

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WEB SITE

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http://www.users.kudpc.kyoto-u.ac.jp/~p51907/index-e.html