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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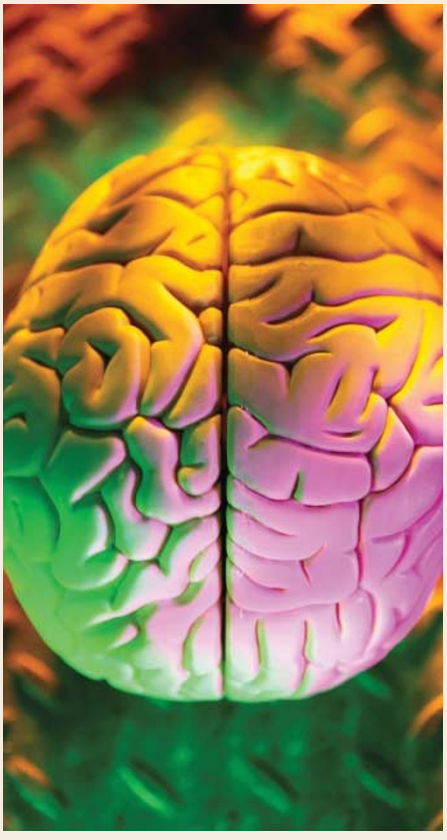

SIGNAL TRANSDUCTION

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.

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