Microtubule cytoskeleton: **No longer an also Ran**

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The Ran GTPase cycle has been extensively studied in the context of nuclear transport. Recent work indicates that this GTPase cycle also plays an important role in regulating the microtubule cytoskeleton.

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The microtubule cytoskeleton plays a central role in cell division, cytoplasmic organization, the establishment and maintenance of cellular asymmetry and in some forms of cell motility. The varied functions of the microtubule cytoskeleton imply that both intracellular and extracellular signal transduction pathways regulate its organization and dynamic properties; the identity of these pathways remains largely unknown, however. The most obvious and widely conserved change in the microtubule cytoskeleton is its dramatic reorganization during the transition from interphase to mitosis. Central to the molecular analysis of this transition has been the development of extracts from unfertilized *Xenopus* eggs that recapitulate many aspects of the transition *in vitro* [1]. These extracts are an excellent general experimental system for analyzing the regulation of the microtubule cytoskeleton — for identifying and characterizing direct regulators of microtubule polymer ization dynamics as well as for dissecting signal-dependent changes in the microtubule cytoskeleton. The experimental attractiveness of these extracts is evident in several recent papers in which the extracts are used to demonstrate an unexpected role for the Ran GTPase in regulating the microtubule cytoskeleton.

Ran GTPases represent one branch of the Ras superfamily of small GTPases. The Ras GTPases use the energy of GTP hydrolysis to generate a molecular switch in a signal transduction pathway. These small GTPases cycle between an active GTP-bound state and an inactive GDP-bound state; the conversion between these two states is aided by accessory factors such as exchange factors and GTPase activator proteins (GAPs). Different Ras-superfamily GTPases interact in their active GTP-bound states with different effector proteins to regulate a variety of intracellular processes [2]. To date, the Ran GTPase cycle has been primarily implicated in nuclear transport [3]. Differences in the ratio of Ran–GTP: Ran–GDP between the nucleus and the cytoplasm are thought to underlie the vectorial nature of nuclear transport. Additional roles for the Ran GTPase cycle have long been suspected, but the difficulty of excluding indirect effects arising from defects in nuclear transport has hampered their investigation. In a key breakthrough last year, Nakamura *et al.* [4] identified a human Ran–GTP binding protein, RanBPM, that localizes to centrosomes — the microtubule organizing centers of animal cells — and appears to play a role in microtubule nucleation. RanBPM overexpression in mammalian tissue culture cells results in the formation of ectopic foci that are active centers for microtubule nucleation. Both RanBPM and γ-tubulin, a component essential for microtubule nucleation, localize to the centers of these ectopic foci. These findings suggested a possible role for the Ran GTPase cycle and its effectors in regulating the microtubule cytoskeleton.

This suggestion has been tested in a series of recent papers in which the effects of directly manipulating the levels of Ran–GTP and Ran–GDP in *Xenopus* egg extracts have been analyzed [5–9]. Nuclear assembly does not occur around exogenously added, demembranated sperm nuclei in the metaphase-arrested extracts, circumventing potential indirect effects arising from defects in nuclear transport. All the papers describe schemes to alter the ratio of Ran–GTP : Ran–GDP that rely on manipulating specific components of the Ran GTPase cycle (Figure 1a); microtubule polymerization, organization and spindle assembly are then analyzed in the manipulated extracts. The high degree of experimental control possible with small GTPases is evident in the remarkable range of schemes used by the different groups to manipulate Ran activity in the extract. These schemes include the addition of mutant versions of Ran; the addition of wild-type recombinant Ran pre-loaded *in vitro* with different guanine nucleotides; the addition or removal of RCC1, the Ran exchange factor that promotes Ran–GTP formation (Figure 1a); the addition of excess RanBP1, a protein that has high affinity for Ran–GTP and that — together with the Ran GTPase activator RanGAP — stimulates hydrolysis of GTP bound to Ran (Figure 1a); and various combinations of these schemes.

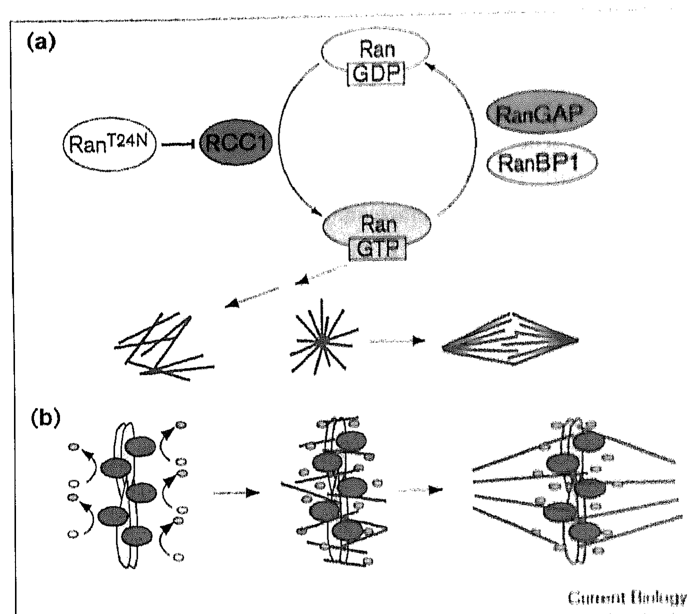
Despite some differences in the details, the general conclusion of all five studies is that the Ran GTPase cycle regulates microtubule assembly in *Xenopus* egg extracts. In a normal metaphase-arrested extract, addition of a demembranated sperm nucleus triggers the aggregation of active nucleating material around its basal body and formation of a functional centrosome that goes on to become a microtubule aster. In the absence of added sperm nuclei,

microtubule asters do not form. When Ran–GTP levels are increased experimentally, however, large numbers of free microtubule asters form in the absence of sperm nuclei [5–8] (Figure 1a). How might Ran–GTP promote the formation of free microtubule asters? One possibility is that Ran–GTP promotes the activation and aggregation of microtubule-nucleating material. Microtubule polymerization from such aggregates would result in aster formation. A second possibility is that elevated levels of Ran–GTP promote free microtubule assembly in the extract, and motor proteins, particularly cytoplasmic dynein, organize these free microtubules into asters. Addition of antibodies that inhibit dynein prevents the organization of microtubules into asters — an observation that supports the second possibility — although the persistence of microtubule bundles focused at one end when dynein is inhibited suggests that both mechanisms may act in concert [6,7].

What is the specific mechanism by which Ran–GTP stimulates microtubule assembly? Preliminary analysis suggests that, as expected for a GTPase signaling molecule, Ran does not directly influence tubulin polymerization; presumably, Ran effector proteins execute this function [7]. One possible mechanism to explain the effects observed in extracts is that Ran–GTP effectors promote microtubule nucleation; that is, they help overcome the kinetic barrier to the formation of new microtubule ends [10]. A second possible mechanism is that Ran–GTP effectors alter the dynamic properties of existing microtubule ends. Polymerizing and depolymerizing microtubules coexist and infrequently interconvert — a non-equilibrium behavior termed 'dynamic instability' [11]. Ran–GTP effectors could promote microtubule assembly by influencing factors that regulate any of the parameters of dynamic instability.

A comparison of the experiments performed by the different groups offers some hints about the mechanism of Ran action, although much more work is needed to resolve this issue. Kalab *et al.* [5] find that increasing Ran–GDP by adding excess RanBP1 inhibits the formation of the sperm microtubule aster. Carazo-Salas *et al.* [8] find that increasing Ran–GDP by adding Ran^{T24N}, a Ran mutant that binds and inhibits the activity of the RCC1 exchange factor, does not affect the length or morphology of microtubule asters assembled onto purified mammalian centrosomes in extracts. Assuming that both of these perturbations act solely to elevate Ran–GDP levels, the differences observed on the different substrates used to assay aster formation are revealing. In the former case, assembly of activated microtubule-nucleating material onto the sperm basal body is a prerequisite for microtubule aster formation; in the latter case, a centrosome containing pre-assembled active microtubule-nucleating material is used to assay microtubule aster formation. Thus, these different results suggest that Ran–GTP acts to influence

Figure 1



(a) A simplified view of the Ran GTPase cycle. RCC1 is a Ran guanine-nucleotide exchange factor that promotes Ran–GTP formation by exchanging GDP bound to Ran with GTP. The Ran^{T24N} mutant indirectly promotes Ran–GDP formation by binding to RCC1 and inhibiting its exchange activity. RanGAP is a Ran GTPase activator that stimulates hydrolysis of GTP bound to Ran. RanBP1 promotes Ran–GDP formation by increasing the rate of RanGAP-mediated Ran GTP hydrolysis (for details, see [3]). Increasing Ran–GTP levels in metaphase-arrested *Xenopus* egg extracts signals the formation of microtubule asters and spindle-like structures in the absence of centrosomes and chromosomes. Although the effectors of Ran–GTP that signal microtubule assembly in *Xenopus* extracts are not yet known, RanBPM, a mammalian Ran–GTP-binding protein that localizes to centrosomes, is an attractive candidate [4]. (b) A model for the role of RCC1 localization in chromatin-driven spindle assembly [8]. Chromatin-associated RCC1 is postulated to generate a locally high concentration of Ran–GTP that induces microtubule polymerization in the vicinity of chromatin; subsequent reorganization by motor proteins generates bipolar spindles.

assembly or activity of microtubule-nucleating material and not to alter the dynamics of existing microtubule ends. The centrosomal localization of RanBPM and its binding specificity for Ran–GTP are consistent with this hypothesis. Interestingly, stimulation of microtubule assembly by Ran–GTP requires a microtubule-nucleating complex termed γ-TuRC, which contains γ-tubulin and is required for normal microtubule nucleation in egg extracts [12]. Thus, Ran–GTP does not act by promoting γ-TuRC-independent nucleation. In addition to directly manipulating RanBPM in egg extracts, determining the mechanism by which Ran–GTP stimulates microtubule assembly will require measuring the parameters of microtubule dynamic instability in extracts where the ratio of Ran–GTP: Ran–GDP has been manipulated.

What is the role of the Ran GTPase cycle in the regulation of the microtubule cytoskeleton *in vivo*? One possibility is

that the Ran GTPase cycle regulates microtubule polymerization during spindle assembly. This might be particularly important in systems where spindle assembly is directed by chromatin, such as in some meiotic systems and exemplified by *Xenopus* egg extracts. In 'traditional' mitotic spindle assembly pathways, microtubules polymerized from centrosomes are thought to associate with chromosomes to generate a bipolar spindle [13]. In *Xenopus* extracts, however, bipolar spindles can assemble around chromatin in the absence of centrosomes. This type of spindle-assembly mechanism has been studied by adding DNA-coated beads to extracts [14]. During the initial stages of spindle assembly around these DNA beads, a cloud of microtubules appears in the proximity of the beads. These microtubules subsequently reorganize around the beads to form a spindle with focused poles. Because no centrosomes are present, such a spindle assembly pathway involves the preferential *de novo* assembly of microtubules in the vicinity of chromatin.

The association of the Ran exchange factor RCC1 with chromatin is thought to establish the high Ran–GTP concentration in the nucleus relative to the cytoplasm that is observed during interphase [3]. Carazo-Salas *et al.* [8] propose that this localization of RCC1 might increase Ran–GTP levels in the vicinity of chromatin and thereby induce microtubule polymerization preferentially in the vicinity of chromatin during spindle assembly around DNA beads (Figure 1b). Inhibiting RCC1 with the Ran^{T24N} mutant blocks spindle assembly around DNA beads, supporting a role for RCC1 activity in this process. This hypothesis predicts that manipulating Ran–GTP levels to perturb the postulated high Ran-GTP concentration localized in the vicinity of chromatin should also perturb spindle assembly around DNA beads. Consistent with this prediction, increasing Ran–GTP globally in the extract partially 'uncouples' microtubule assembly from chromatin beads; that is, preferential microtubule assembly around DNA beads is reduced, most microtubule assemblies are independent of the beads, and bead clusters induce spindle assembly less effectively than in unperturbed extracts [8]. When Ran–GTP levels are increased to a more modest extent — an extent insufficient to induce bead-independent microtubule assembly — spindle assembly around DNA beads is enhanced and small bead clusters, normally incapable of forming spindles, now support spindle assembly. This observation is interpreted to mean that a small global increase in Ran–GTP can enable lower levels of chromatin-associated RCC1 activity to generate enough Ran–GTP to induce local microtubule assembly [8].

The results from these DNA bead experiments lead to the model that local generation of Ran–GTP by RCC1 around chromatin may be the mechanism for inducing local microtubule assembly during chromatin-driven spindle assembly. Although this is an attractive and exciting hypothesis, there is an alternative explanation for the findings of Carazo-Salas *et al.* The experiments presented do not directly show that Ran controls microtubule assembly in the vicinity of chromatin, but rather show that Ran controls microtubule dynamics globally in the extract and perturbing this control affects spindle assembly. High levels of Ran–GTP promote microtubule assembly throughout the extract, perhaps depleting essential assembly-promoting components, and thereby 'uncoupling' microtubule assembly from the DNA beads. The observation that modestly increased Ran–GTP levels lower a threshold for spindle assembly around DNA beads may be explained in a similar way. It is possible that similar results might be obtained by adding appropriate concentrations of agents such as taxol or DMSO that have a general stimulatory effect on microtubule assembly in extracts.

One way to exclude this possibility, as well as to test directly the requirement for high local Ran–GTP levels in chromatin-driven spindle assembly, would be to generate variable levels of cytoplasmic Ran–GTP in the absence of chromatin-associated RCC1. This would require first depleting RCC1 from the extract, then adding variable amounts of Ran–GTP and assaying spindle assembly around DNA beads. If spindle assembly on the beads occurs at some Ran–GTP level in the absence of RCC1, and hence in the absence of a locally high Ran–GTP environment in the proximity of the beads, then the localized Ran–GTP hypothesis discussed above seems untenable. If spindle assembly around DNA beads cannot occur at any concentration of exogenous Ran–GTP, then the evidence for an RCC1-dependent local increase in Ran–GTP driving the initial burst of microtubule polymerization during chromatin-driven spindle assembly becomes more compelling.

In addition to a role for Ran in microtubule assembly, these initial studies suggest that it may have a role in the generation of spindle bipolarity. Microtubules polymerized in extracts with high levels of Ran–GTP reorganize into asters — a result that is expected on the basis of earlier work with microtubule-stabilizing agents [15]. More remarkably, microtubules also assemble into bipolar spindle-like structures (Figure 1a). The ability to self-organize bipolar structures in the absence of centrosomes and chromosomes has not been previously demonstrated in egg extracts but has been documented in enucleated mouse oocytes [16]. These results may represent additional effects of Ran, perhaps on the motor proteins that influence spindle bipolarity, or may reveal hitherto unforeseen principles underlying the establishment of spindle bipolarity.

Does the Ran GTPase cycle play a role in spindle assembly in somatic mitotic cells? Nucleoplasm containing high

levels of Ran–GTP that would be released following nuclear envelope breakdown could play a role in the early stages of spindle assembly. However, it has been reported that spindles can assemble in the absence of RCC1 in somatic cells: a hamster cell line with a temperature-sensitive RCC1 mutation shifted to the non-permissive temperature during S-phase rapidly loses RCC1 protein, prematurely enters mitosis, and forms a bipolar mitotic spindle [17]. At first glance, this observation argues against a role for RCC1 activity in spindle assembly in somatic mammalian cells, but this issue needs to be more thoroughly investigated. A possible alternative physiological role of the Ran GTPase cycle may be at the transition from mitosis to interphase, when residual spindles are disassembled and the interphase microtubule network is formed. Consistent with this idea, increasing the levels of Ran–GTP prevents normal breakdown of the sperm microtubule aster in interphase *Xenopus* egg extracts [9].

Cumulatively, these initial studies decisively implicate the Ran GTPase cycle in the regulation of the microtubule cytoskeleton and provide a plethora of new directions for future studies that are bound to yield new insights, not only into the physiological roles of the Ran GTPase cycle, but also into the cellular functions of the microtubule cytoskeleton.

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