# Spindles get the Ran around

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*Despite its fundamental role in cell division, the mitotic spindle remains an enigmatic figure in cell biology. This is due to the complex dynamic behaviour of microtubules, which form the spindle fibres responsible for segregating chromosomes to opposite ends of the cell during mitosis. Recent reports indicate that the small GTPase Ran, which plays a key role in nuclear transport, also has a role in mitosis by regulating microtubule nucleation and/or growth. The race is now on to determine how Ran exerts its effects on spindle assembly.*

Viewed by its fans as the quintessential macromolecular apparatus, the mitotic spindle carries out the vital task of dividing duplicated chromosomes into two complete sets prior to cytokinesis. At the onset of mitosis in most animal cells, the nuclear envelope and interphase microtubule array disassemble and the duplicated centrosomes nucleate microtubules that interact with chromosomes, forming a bipolar spindle. Sister chromosomes balanced at the spindle equator segregate and move to opposite spindle poles during anaphase. A major goal in spindle research is to understand how microtubules are regulated in mitosis to generate their polarized orientation in the spindle. In recent years, much progress has been made in understanding the fundamental principles of microtubule dynamics, their regulation by stabilizing/destabilizing factors and their organization by motor proteins (for reviews see Refs 1–3). However, we still come up short when we try to explain how these factors are regulated and coordinated to promote spindle assembly and function. In particular, it is not yet understood how chromosomes contribute to microtubule stabilization and organization[4,5]. Recently, an important missing link might have been identified with the discovery that the small GTPase Ran can regulate microtubule nucleation and stability during mitosis. This article summarizes new findings and attempts to put them in the context of current models of spindle assembly.

## The multiple personalities of Ran

The highly conserved GTPase Ran, like other members of the Ras superfamily, acts as a switch, interacting with distinct sets of proteins in its GTP- or GDP-bound state[6]. Ran itself is an inefficient GTPase. Consequently, its nucleotide state is dictated by a GTPase-activating protein (GAP) and a GDP–GTP exchange factor (GEF) that promote the formation of RanGDP and RanGTP, respectively (Fig. 1). Ran is an abundant protein, found mainly in the nucleus at steady state. However, the regulators of the Ran cycle are highly compartmentalized. The GAP for Ran, RanGAP, is restricted to the cytoplasm, whereas RCC1, the RanGEF, is a nuclear protein that binds to chromatin[7]. This asymmetric distribution is crucial for the directionality of transport through the nuclear pore because Ran regulates the interaction between nuclear transport receptors and their cargoes[6]. RCC1 dominates in the nucleus, where Ran in its GTP-bound form causes disassembly of nuclear import complexes and assembly of nuclear export complexes. Once transported to the cytoplasm through nuclear pores, export complexes are disassembled and release their cargo owing to GTP hydrolysis induced by RanGAP, which is further accelerated by cytoplasmic Ran-binding proteins such as RanBP1. Subsequently, RanGDP can re-enter the nucleus, where it is converted back to RanGTP by RCC1, starting the cycle over again. Hence, RanGTP acts as a positional marker that defines the nucleoplasm for nucleocytoplasmic transport events.

Aside from its well-documented role in nuclear transport, the Ran system has been implicated in a variety of cellular activities, including cell-cycle progression, DNA replication, nuclear and chromosomal architecture and RNA processing[8]. A role for the Ran cycle in spindle assembly has been suggested by several experiments. For example, overexpression of the RCC1 homologue in yeast specifically suppresses a class of α-tubulin mutations that arrest as large budded cells with excess microtubules[9]. However, it has been difficult to distinguish whether effects of Ran disruption are indirect, arising from the disruption of nucleocytoplasmic transport. More-compelling was the recent identification of RanBPM, a Ran-binding protein localized to centrosomes in mammalian cells[10]. RanBPM was identified in a two-hybrid screen with Ran but has no homology to other previously identified Ran-interacting proteins. Its overexpression leads to the formation of ectopic microtubule asters containing γ-tubulin, suggesting that Ran could influence microtubule assembly.

## Frog power

The key to identifying a mitotic role for Ran was the use of extracts from eggs of the African frog *Xenopus laevis*. Extracts prepared from eggs arrested in metaphase can be used for spindle-assembly reactions without transiting through the cell cycle, thereby circumventing any requirement for nucleus formation and nuclear transport. Demembranated sperm nuclei added to mitotic extracts nucleate microtubule asters as the associated centriole recruits microtubule-nucleating factors such as γ-tubulin. Microtubules polarize their growth towards sperm chromatin, forming 'half spindles' that fuse pairwise to form bipolar structures (Fig. 2a,b). Several groups have exploited this system, and, with only minor

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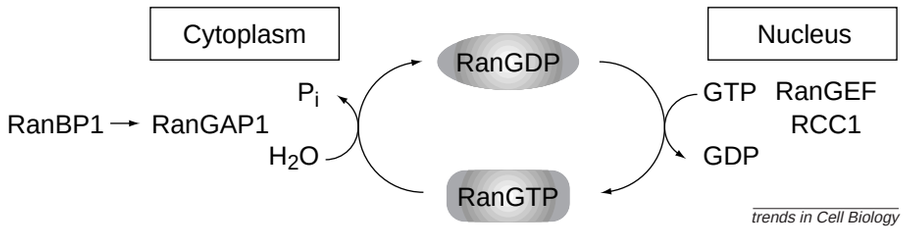


**FIGURE 1**

The Ran nucleotide cycle. RanGDP is converted to RanGTP by the GDP–GTP exchange factor (GEF) RCC1 in the nucleus. In the cytoplasm, Ran hydrolyses GTP and converts to the GDP-bound form, in a reaction that is promoted by RanGAP and the accessory protein RanBP1. The compartmentalized distribution of the Ran regulators ensures that RanGTP is highly enriched inside the nucleus.

discrepancies, all of them show that Ran in its GTP-bound form promotes the formation of microtubules in a mitotic cytoplasm[11–15]. This was established by manipulating factors that affect the levels of RanGTP or by using bacterially expressed Ran mutants that have defects in nucleotide hydrolysis or exchange.

The first report, from the laboratory of Mary Dasso[11], showed that addition of RanBP1, which acts as a cofactor for RanGAP by increasing the rate of RanGTP hydrolysis, dramatically inhibited microtubule polymerization at sperm centrosomes in the extract. By contrast, addition of RCC1 promoted microtubule assembly, suggesting that generation of RanGTP is required for spindle assembly. Interestingly, increasing the levels of RanGTP in extracts appeared to uncouple microtubule assembly from chromosomes, resulting in ectopic microtubule asters that frequently fused to form bipolar or multipolar spindle-like structures in the absence of chromosomes or centrosomes (Fig. 2d). That RanGTP can stimulate microtubule assembly was supported by the use of two Ran mutants (Table 1). RanT24N is unable to bind to GTP and inhibits the exchange activity of RCC1, thus interfering with the formation of RanGTP. By contrast, RanG19V bound to GTPγS is unable to undergo RanGAP-stimulated GTP hydrolysis, thereby increasing the RanGTP concentration. As predicted, these mutants had opposite effects on microtubule assembly. RanT24N inhibited assembly, whereas RanG19V promoted formation of ectopic asters and spindles.

These exciting results showed that manipulation of Ran by exogenously added factors could affect microtubule dynamics, but is the Ran system required for spindle assembly? Two additional papers (published back-to-back in *Science*) supported and extended the Ran–microtubule connection[12,13]. Ohba *et al*. demonstrated a physiological role for RCC1 by showing that immunodepletion of the protein blocked microtubule assembly in the extract, which could be rescued by adding back the bacterially expressed and purified protein. However, addition of an RCC1 mutant defective in RanGEF activity could not rescue, indicating that RanGTP levels regulate microtubule polymerization. Wilde and Zheng found that a different Ran mutant, RanL43E, was particularly potent in leading to formation of ectopic microtubule asters and chromatin-free spindles. This mutant locks the protein in the GTP-bound state and is also thought to affect interactions with downstream effectors (Table 1). Both groups showed that Ran-induced structures contained epitopes found at spindle poles: NuMA and the γ-tubulin ring complex. Like wild-type spindle poles, focusing of microtubule minus-ends in the Ran-induced structures was dependent on the activity of the microtubule-based motor cytoplasmic dynein. Wilde and Zheng reported that RanGTP did not stimulate the polymerization of purified α–β tubulin into microtubules, indicating that Ran must regulate cellular factors in the extract to stimulate microtubule polymerization. Furthermore, immunodepletion of the γ-tubulin ring complex or the spindle microtubule-associated XMAP215 inhibited Ran aster formation as severely as sperm spindle assembly, indicating that the formation of Ran structures requires the same complement of cellular factors as is required for normal spindle assembly[16,17].

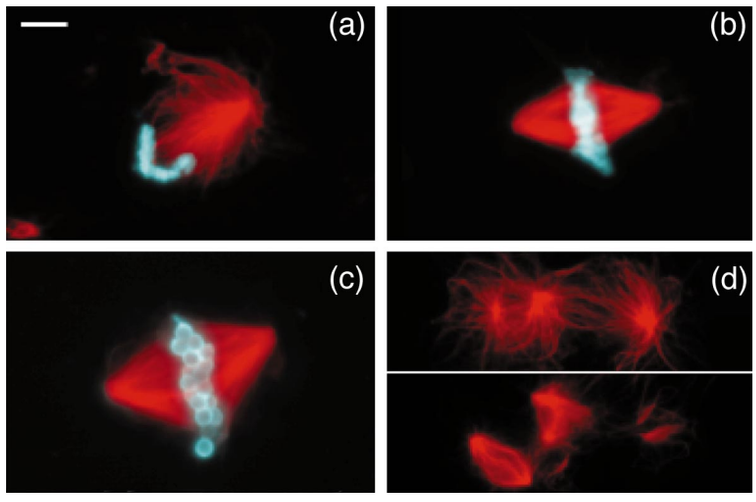


**FIGURE 2**

Mitotic figures assembled in *Xenopus* egg extracts. Microtubules are red and DNA is blue. (a) Sperm DNA spindle intermediate with centrosome nucleating microtubules polarized towards chromosomes. (b) Bipolar sperm DNA spindle. (c) Bipolar spindle assembled around DNA-coated beads in the absence of centrosomes. (d) Astral and spindle-like figures formed in extracts in the presence of the RanQ69L-mutant loaded with GTP (see Table 1). Bar, 10 μm.

## Ran and the chromosome connection

How does a role for Ran fit into models of spindle assembly? The connection is thought to be at the level of chromosomes. Several lines of research indicate that, following nuclear envelope breakdown, nuclear or chromosomal factors stimulate microtubule polymerization and promote spindle organization. The extreme case is evident in female meiotic cells lacking centrosomes. In these cells, microtubules nucleate specifically around chromosomes, then self-organize in a motor-dependent

fashion into a bipolar spindle[18,19]. Chromosomal influence on spindle assembly is also apparent in some cells containing centrosomes. In grasshopper spermatocytes, microtubule density in the spindle correlates with the number and proximity of chromosomes[20]. Premature rupture of the nuclear envelope in prophase causes precocious spindle assembly around the exposed chromosomes[21].

A *Xenopus* model system for meiotic-style spindle assembly uses small magnetic beads coated with plasmid DNA instead of sperm nuclei[22]. The DNA on the beads assembles into chromatin in the egg extract and induces the formation of bipolar spindles in the absence of centrosomes (Fig. 2c). Using this system, Carazo-Salas *et al.* explored the role of Ran in chromatin-induced spindle assembly[14]. They showed that RCC1 is bound to the chromatin beads and that microtubule growth around the beads is inhibited by addition of RanT24N or RanBP1/RanGAP, factors that would inhibit formation of RanGTP. A model interpreting these experiments and those described above is shown in Fig. 3. An asymmetric distribution of the Ran nucleotide state is created in mitosis, in the absence of a nuclear envelope, by chromosomal RCC1 generating RanGTP locally in the surrounding cytoplasm. As it diffuses away, GTP hydrolysis is promoted by RanGAP. The high local concentration of RanGTP around chromosomes then promotes microtubule polymerization and motor-dependent organization into a spindle. As in nuclear transport, RanGTP functions as spatial cue, but in mitosis it also marks the environment around chromosomes. This model is consistent with observations that a uniform increase in RanGTP concentration uncouples microtubule assembly from chromatin, leading to ectopic microtubule asters and spindles.

**TABLE 1 – MUTATIONS IN THE RAN-GTPase**

| Mutation | Phenotype | Refs |
|---|---|---|
| RanG19V | Deficient in GTP hydrolysis. GTPase activity is not stimulated by RanGAP. Locked in GTP-bound state. | 28,29 |
| RanQ69L | Unable to hydrolyse GTP. GTPase activity is not stimulated by RanGAP. Locked in GTP-bound state. | 30 |
| RanL43E | GTPase activity is not stimulated by RanGAP. Reduced affinity for effectors such as importin β and RanBP1. Locked in GTP-bound state. | 31 |
| RanT24N | Mutant protein is unable to bind to GTP but also has reduced affinity for GDP. Binds tightly to RCC1 and inhibits its GEF activity. | 29,30 |

## Mechanisms of Ran action!?

How does RanGTP exert its effects? At present there are few clues. The overall change in microtubule dynamics at the onset of mitosis is thought to result from activation of the cyclin-dependent kinase cdc2[23]. Many spindle- and microtubule-regulatory proteins subsequently are phosphorylated. Therefore, until now, phosphorylation–dephosphorylation reactions were thought to be the primary regulatory forces generating the spindle. It is not yet known how Ran fits into this phosphorylation cascade. Perhaps, Ran acts in a manner analogous to the small GTPases Rac, Rho and Cdc42, which interact with kinases and other proteins that alter the dynamics of actin polymerization[24]. Based on its switch-like mechanism regulating nuclear transport, it seems likely that Ran interacts with different partners depending on its nucleotide state in mitosis, just as it does in interphase. What are these partners and how might they affect spindle assembly? One obvious candidate is the centrosomal RanBPM, but others must be soluble or associated with the spindle. Intriguing are reports that components of the Ran system, including RanGAP1[25] and Ran itself[15], are localized to spindles, suggesting that the Ran-hydrolysis cycle could regulate microtubules directly through spindle-associated proteins, either by activating stabilizing factors or by inactivating inhibitors.

Does the mitotic role for Ran have any relationship with its interphase role in nuclear transport? Interestingly, two conserved factors involved in yeast nuclear export, Chromosome Segregation 1 (Cse1p) and Chromosomal Region Maintenance 1 (Crm1p) originally were identified as having defects in chromosome structure and/or segregation[26,27]. It will be interesting to determine whether other transport factors also have dual roles or whether they regulate the association of Ran with mitotic factors. It also needs to be established whether the effects of Ran on spindle assembly in *Xenopus* egg extracts can be reproduced in somatic cells. Finally, it remains to be elucidated how the interphase and

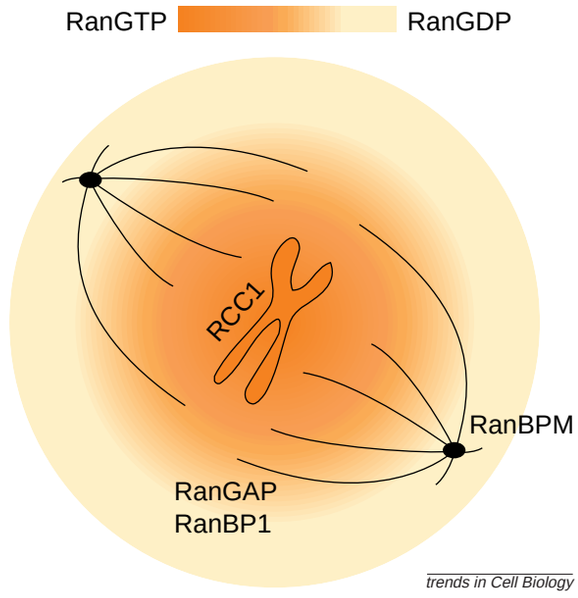


**FIGURE 3**

Model showing the proposed effects of chromosomal RCC1 on RanGTP/RanGDP levels in the surrounding cytoplasm. GDP–GTP exchange factor (GEF) RCC1 converts RanGDP to RanGTP close to chromosomes, while cytoplasmic RanGAP together with RanBP1 leads to GTP hydrolysis by Ran, generating a gradient of RanGTP that promotes microtubule nucleation and growth towards chromosomes.

mitotic functions of the Ran system tie into its apparent roles in cell-cycle progression and the DNA-replication checkpoint. Does Ran also play a direct role in other cellular processes independent of transport? Future studies of the role of Ran in spindle assembly promise to give insight into the mechanisms of action of this truly interesting protein.

## References


1 Hyman, A.A. and Karsenti, E. (1996) Morphogenetic properties of microtubules and mitotic spindle assembly. *Cell* 84, 401–410

2 Desai, A. and Mitchison, T.J. (1997) Microtubule polymerization dynamics. *Annu. Rev. Cell. Dev. Biol.* 13, 83–117

3 Walczak, C.E. *et al.* (1998) A model for the proposed roles of different microtubule-based motor proteins in establishing spindle bipolarity. *Curr. Biol.* 8, 903–913

4 Hyman, A. and Karsenti, E. (1998) The role of nucleation in patterning microtubule networks. *J. Cell Sci.* 111, 2077–2083

5 Andersen, S.S. (1999) Balanced regulation of microtubule dynamics during the cell cycle: a contemporary view. *BioEssays* 21, 53–60

6 Mattaj, I.W. and Englmeier, L. (1998) Nucleocytoplasmic transport: the soluble phase. *Annu. Rev. Biochem.* 67, 265–306

7 Dasso, M. (1993) RCC1 in the cell cycle: the regulator of chromosome condensation takes on new roles. *Trends Biochem. Sci.* 18, 96–101

8 Rush, M.G. *et al.* (1996) The small nuclear GTPase Ran: how much does it run? *BioEssays* 18, 103–112

9 Kirkpatrick, D. and Solomon, F. (1994) Overexpression of yeast homologs of the mammalian checkpoint gene RCC1 suppresses the class of alpha-tubulin mutations that arrest with excess microtubules. *Genetics* 137, 381–392

10 Nakamura, M. *et al.* (1998) When overexpressed, a novel centrosomal protein, RanBPM, causes ectopic microtubule nucleation similar to gamma-tubulin. *J. Cell Biol.* 143, 1041–1052

11 Kalab, P. *et al.* (1999) The ran GTPase regulates mitotic spindle assembly. *Curr. Biol.* 9, 481–484

12 Ohba, T. *et al.* (1999) Self-organization of microtubule asters induced in *Xenopus* egg extracts by GTP-bound Ran. *Science* 284, 1356–1358

13 Wilde, A. and Zheng, Y. (1999) Stimulation of microtubule aster formation and spindle assembly by the small GTPase Ran. *Science* 284, 1359–1362

14 Carazo-Salas, R.E. *et al.* (1999) Generation of GTP-bound Ran by RCC1 is required for chromatin-induced mitotic spindle formation. *Nature* 400, 178–181

15 Zhang, C. *et al.* (1999) Ran-GTP stabilises microtubule asters and inhibits nuclear assembly in *Xenopus* egg extracts. *J. Cell Sci.* 112, 2453–2461

16 Joshi, H.C. *et al.* (1992) Gamma-tubulin is a centrosomal protein required for cell cycle- dependent microtubule nucleation. *Nature* 356, 80–83

17 Cha, B.J. *et al.* (1998) XMAP230 is required for the assembly and organization of acetylated microtubules and spindles in *Xenopus* oocytes and eggs. *J. Cell Sci.* 111, 2315–2327

18 Theurkauf, W.E. and Hawley, R.S. (1992) Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.* 116, 1167–1180

19 Gard, D.L. (1992) Microtubule organization during maturation of *Xenopus* oocytes: assembly and rotation of the meiotic spindles. *Dev. Biol.* 151, 516–530

20 Zhang, D. and Nicklas, R.B. (1995) The impact of chromosomes and centrosomes on spindle assembly as observed in living cells. *J. Cell Biol.* 129, 1287–1300

21 Zhang, D. and Nicklas, R.B. (1995) Chromosomes initiate spindle assembly upon experimental dissolution of the nuclear envelope in grasshopper spermatocytes. *J. Cell Biol.* 131, 1125–1131

22 Heald, R. *et al.* (1996) Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* egg extracts. *Nature* 382, 420–425

23 Verde, F. *et al.* (1992) Control of microtubule dynamics and length by cyclin A- and cyclin B-dependent kinases in *Xenopus* egg extracts. *J. Cell Biol.* 118, 1097–1108

24 Schmidt, A. and Hall, M.N. (1998) Signaling to the actin cytoskeleton. *Annu. Rev. Cell Dev. Biol.* 14, 305–338

25 Matunis, M.J. *et al.* (1996) A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J. Cell Biol.* 135, 1457–1470

26 Xiao, Z. *et al.* (1993) CSE1 and CSE2, two new genes required for accurate mitotic chromosome segregation in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 13, 4691–4702

27 Adachi, Y. and Yanagida, M. (1989) Higher order chromosome structure is affected by cold-sensitive mutations in a *Schizosaccharomyces pombe* gene *crm1*$^{+}$ which encodes a 115-kD protein preferentially localized in the nucleus and its periphery. *J. Cell Biol.* 108, 1195–1207

28 Ren, M. *et al.* (1994) Effects of mutant Ran/TC4 proteins on cell cycle progression. *Mol. Cell. Biol.* 14, 4216–4224

29 Dasso, M. *et al.* (1994) A mutant form of the Ran/TC4 protein disrupts nuclear function in *Xenopus laevis* egg extracts by inhibiting the RCC1 protein, a regulator of chromosome condensation. *EMBO J.* 13, 5732–5744

30 Klebe, C. *et al.* (1995) Interaction of the nuclear GTP-binding protein Ran with its regulatory proteins RCC1 and RanGAP1. *Biochemistry* 34, 639–647

31 Lounsbury, K.M. *et al.* (1996) Mutations within the Ran/TC4 GTPase. Effects on regulatory factor interactions and subcellular localization. *J. Biol. Chem.* 271, 32834–32841


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## New editorial board member

We are delighted to announce that we have recently appointed David Drubin as a new member of the *trends in* CELL BIOLOGY editorial board.

David carried out his PhD research with Marc Kirschner at UCSF and studied the regulation of microtubule assembly by tau protein during neuronal differentiation. For his postdoctoral studies, he joined David Botstein's laboratory at MIT, where he combined biochemical and genetic approaches to the study of the yeast actin cytoskeleton. Since 1988, he has been on the faculty of the Dept of Molecular and Cell Biology at UC Berkeley. He continues to employ biochemical, genetic and cell-biological techniques to elucidate fundamental mechanisms of the regulation of actin assembly and the molecular pathways responsible for cellular morphogenesis and plasma membrane turnover in budding yeast. A particular interest is in the mechanisms that couple signal-transduction pathways to the actin cytoskeleton. Recently, his laboratory has begun to apply principles gleaned from studies in yeast to mammalian cells grown in culture. A major current emphasis of the yeast and mammalian cell studies is on the role of the actin cytoskeleton in mediating endocytosis.