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COMMENTARY

The Ran decathlon: multiple roles of Ran

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SUMMARY

The Ran GTPase system affects many cellular processes, including the regulation of cell cycle progression, nuclear envelope structure and function, and nucleocytoplasmic transport. The biochemical basis for the involvement of Ran in nuclear import and export has been well documented, but the direct targets of Ran in other cellular processes have not yet been identified. There is, however, mounting evidence that Ran directly affects at least some of these other cellular processes by mechanisms independent of its role in transport. In this Commentary we discuss evidence linking Ran to different aspects of cell function, and how these multiple facets of Ran's activity may relate to each other.

Key words: RanGTPase, Nucleus, Mitosis, Spindle, Septum

INTRODUCTION

Ran is a small, evolutionarily conserved, eukaryotic GTPase of the Ras superfamily that is essential for viability in every organism tested to date. Initial analyses of the consequences of perturbing the Ran GTPase system resulted in a long list of cellular processes that were impaired, including DNA replication, nuclear envelope growth and structure, chromatin structure, cell cycle progression and RNA export (Sazer, 1996). However, it was difficult to determine which processes are direct downstream targets of Ran and which are perturbed indirectly. Furthermore, the arrays of defects are similar but not identical in different organisms. This could reflect either a common Ran function that is manifested differently because of species-specific physiological differences, or species-specific roles for the Ran GTPase itself. In 1993, two groups identified Ran as a protein required for nuclear protein import in vitro (Melchior et al., 1993; Moore and Blobel, 1993). This finding led to the proposal that nuclear transport is the primary function of the Ran GTPase system and that the other cellular defects observed when Ran is perturbed could be attributed to a failure of nuclear trafficking. This idea became a dominant paradigm in the Ran field for two reasons: first, there is a persuasive simplicity to the idea that Ran has a single primary function; second, this hypothesis was difficult to refute experimentally, because many of the processes in which Ran had been implicated require ongoing nucleocytoplasmic transport. However, recent observations have shown that Ran is indeed a complex and multi-functional protein that has transport-independent effects. Here we discuss the multiple roles of Ran, revisit the question of how many downstream targets Ran has, and consider how these functions relate to each other.

I. A BRIEF OVERVIEW OF THE RAN PATHWAY

Ran is one of the most abundant cellular proteins in HeLa cells (Bischoff and Ponstingl, 1991). Like other Ras-family GTPases, it specifically associates with different cellular proteins, depending upon the nucleotide to which it is bound (Fig. 1). A guanine-nucleotide-exchange factor (RanGEF) and a GTPase-activating protein (RanGAP) act on Ran to promote physiological rates of nucleotide exchange and GTP hydrolysis. Because Ran, its regulators and the other proteins with which it interacts have been identified through a variety of experimental approaches, they have been given multiple names. We have included a summary of these names in Table 1, but in the text, we refer to the GTPase by its mammalian name (Ran) and to the other proteins by their functions (e.g. RanGEF and RanGAP).

Ran is a soluble protein located predominantly but not exclusively in the nucleus of eukaryotic cells, RanGAP is cytoplasmic and RanGEF is nuclear (reviewed by Sazer, 1996). This compartmentation leads to three predictions: (1) In order to undergo a complete round of nucleotide binding and hydrolysis, Ran moves between these two cellular compartments; (2) Ran is primarily GTP bound in the nucleus during interphase and GDP bound in the cytoplasm; and (3) there is a steep GTP-Ran gradient across the nuclear envelope. A small GDP-Ran-binding protein called NTF2 (nuclear transport factor 2) is important for maintaining this

Table 1. Core components of the Ran GTPase pathway

Protein	Reported location	Function	Names
Ran	Metazoans: predominantly nuclear during interphase Metazoans: broadly distributed in mitosis Yeast: predominantly nuclear throughout the cell cycle	GTPase	Ran (metazoans) Fyt1p (*S. pombe*) Spi1p (*S. pombe*) Cnr1p, Cnr2p (*S. cerevisiae*) Cst17p (*S. cerevisiae*) Gsp1p, Gsp2p (*S. cerevisiae*)
RanGAP	Metazoans: cytoplasmic during interphase (associated to nuclear pore) Metazoans: spindle associated in mitosis Yeast: cytoplasmic throughout cell cycle, concentrated at the nuclear envelope	GTPase-activating protein	RanGAP1 (vertebrates) Segregation Distorter (*D. melanogaster*) Rna1p (*S. pombe* and *S. cerevisiae*)
RanGEF	Metazoans: nuclear during interphase, associated with chromatin Metazoans: concentrated on chromosomes in mitosis, but not exclusively localized there Yeast: nuclear throughout the cell cycle	Guanine-nucleotide-exchange factor	RCC1 (vertebrates) BJ1 (*D. melanogaster*) Dcd1p (*S. pombe*) Pim1p (*S. pombe*) Mtr1p (*S. cerevisiae*) Prp20p (*S. cerevisiae*) Srm1p (*S. cerevisiae*)
RanBP1	Metazoans: cytoplasmic during interphase Yeast: cytoplasmic throughout the cell cycle	Ran-GTP-binding protein RanGAP accessory factor Promotes release of Ran-GTP from transport receptor complexes	RanBP1 (vertebrates) Sbp1p (*S. pombe*) Cst20p (*S. cerevisiae*) Yrb1p (*S. cerevisiae*)

gradient (see below). In addition to these Ran regulators, three other classes of proteins bind directly to Ran (Fig. 1). One is a group of GTP-Ran-binding proteins that have homology to mammalian RanBP1 (Beddow et al., 1995; Coutavas et al., 1993). RanBP1 or isolated Ran-binding domains from other family members do not have RanGAP activity, but in vitro they can stimulate RanGAP-mediated hydrolysis of GTP bound to Ran approximately tenfold (Bischoff et al., 1995). A second class of GTP-Ran-binding proteins includes importin β and related transport receptors. The association of these proteins with GTP-Ran promotes their assembly or disassembly with transport cargo during nuclear trafficking (see below). Finally, two groups have recently documented that GDP-Ran can associate with zinc-finger domains of nuclear pore proteins (Nakielny et al., 1999; Yaseen and Blobel, 1999), although the function of these interactions has not been established.

II. NUCLEAR TRANSPORT

We treat the role of Ran in nucleocytoplasmic transport briefly here because it has been extensively covered in previous reviews (for instance, Izaurralde and Adam, 1998; Mattaj and Englmeier, 1998). Many importin-β-related GTP-Ran-binding proteins act as transport receptors for either

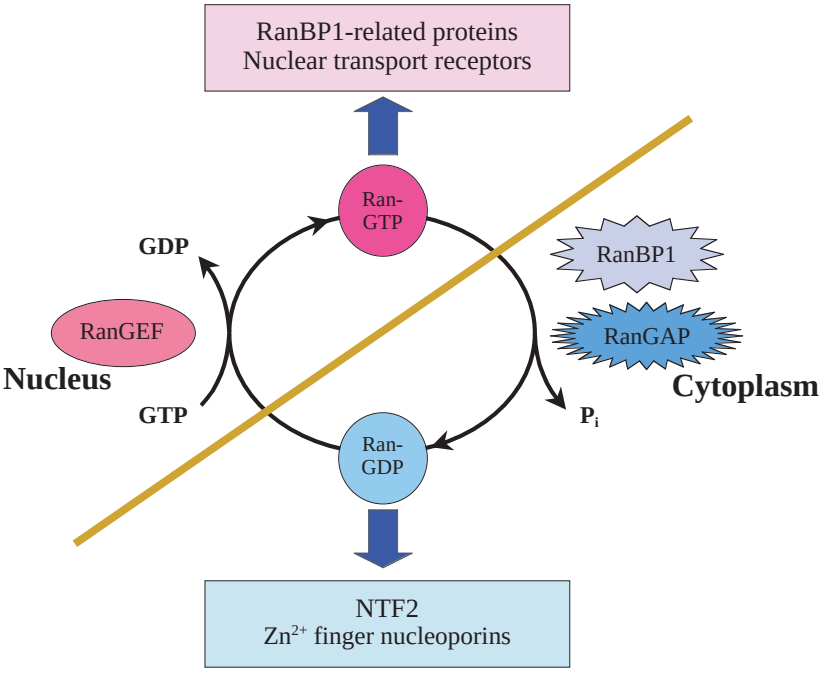


Fig. 1. Ran alternates between its GTP- and GDP-bound forms in a regulated manner. The GTP- and GDP-bound forms of Ran associate with different sets of proteins, which are indicated in the pink and blue boxes, respectively. Guanine-nucleotide exchange is catalyzed by a chromatin-bound nucleotide-exchange factor, RanGEF; GTP hydrolysis is catalyzed by RanGAP in association with RanBP1, an accessory protein. Although RanBP1 can moderately accelerate the rate of RanGAP-mediated nucleotide hydrolysis in the absence of other proteins (Bischoff et al., 1995), RanBP1 might be critical in vivo for nucleotide hydrolysis of Ran-GTP that is bound to nuclear-transport receptors (Bischoff and Gorlich, 1997; Vetter et al., 1999). Nucleotide exchange and hydrolysis factors are compartmentalized in the nucleus and cytoplasm, respectively; this predicts that there is a strong asymmetry of Ran-GTP distribution across the nuclear envelope (indicated by yellow line).

import or export (Mattaj and Englmeier, 1998). Generally, these proteins bind to their substrates directly – the exception being importin β, which requires an adapter subunit (importin α) to associate with its cargo. Both import and export receptors exit the nucleus in association with GTP-Ran. However, import receptors associate with their cargo in the cytosol, where GTP-Ran concentrations are low and dissociate from their cargo upon binding GTP-Ran in the nucleus, whereas export receptors conversely bind their cargo in complexes containing GTP-Ran in the nucleus and release their cargo in the cytosol after GTP hydrolysis. Because the unidirectional trafficking of GTP-Ran out of the nucleus in association with both import and export receptors would dissipate the Ran gradient, cells need a mechanism for re-generating the gradient. Recent evidence suggests that NTF2 plays this role by binding to GDP-Ran in the cytoplasm and facilitating its import into the nucleus, where it encounters RanGEF and undergoes nucleotide exchange (Ribbeck et al., 1998; Smith et al., 1998).

Three aspects of this model are notable. (1) *Conformational changes in Ran resulting from nucleotide binding act as a molecular switch for the assembly and disassembly of protein complexes in a manner that is reminiscent of other Ras family members.* Mutations that block Ran nucleotide exchange or hydrolysis should effectively inhibit all nuclear trafficking despite the fact that these mutants would be predicted to cause accumulation of GDP-Ran or GTP-Ran, respectively. The fact that the phenotypes of RanGEF and RanGAP mutants are remarkably similar is consistent with this prediction (Forrester et al., 1992; Matynia et al., 1996). (2) *This model predicts that the GTP-Ran gradient across the nuclear envelope is essential for nucleocytoplasmic transport and that the directional nature of both import and export are derived from this gradient.* This prediction is supported by in vivo studies showing that the Ran gradient is required for nuclear transport (Izaurralde et al., 1997; Richards et al., 1997) and by experiments using permeabilized cells that confirm the role of the gradient in orienting the direction of transport (Nachury and Weis, 1999). This model, however, predicts that hydrolysis of Ran-bound GTP is not necessarily coupled to translocation of transport-receptor-substrate complexes across the nuclear pore and that the energetic contribution of hydrolysis of Ran-bound GTP to the movement of substrates against a concentration gradient is made at the step of receptor recycling in the cytoplasm. Translocation of transport receptors across the pore is independent of nucleotide hydrolysis (Englmeier et al., 1999; Ribbeck et al., 1999), which is again consistent with this idea. (3) *This model implies that the nucleotide-binding state of Ran can serve as a marker for compartment identity, and that it directs the assembly or disassembly of protein complexes in a manner that is appropriate to the nucleus or cytoplasm.*

Although considerable experimental evidence supports this model, it is fair to say that it has not been definitively proven to be the mechanism of transport. Alternative hypotheses have been proposed in which directionality of movement through nuclear pores is due to sequential binding and release of transport complexes with proteins that line the pores (Rexach and Blobel, 1995). Future investigations should clarify which of these models is correct.

III. MITOTIC PROGRESSION

Several observations have implicated Ran in regulation of the onset of mitosis. This link was first discovered in *ts*BN2 cells, a mutant BHK (baby hamster kidney) cell line that contains a temperature-sensitive allele of *RCC1*, the hamster gene for RanGEF (Nishitani et al., 1991). *ts*BN2 cells that are arrested in S phase by DNA-replication inhibitors enter mitosis prematurely when shifted to the restrictive temperature, despite the presence of unreplicated DNA that would normally block the onset of mitosis. The precocious advancement into M phase is associated with nuclear envelope breakdown (NEB) and dramatic premature chromosome condensation (PCC), as well as full activation of $p34^{cdk1}$-cyclin-B kinase, a key inducer of M phase in all eukaryotes (Nishitani et al., 1991). Together with subsequent studies, these results suggest that *ts*BN2 cells do not block entry into mitosis at the restrictive temperature because they are unable either to recognize or to respond appropriately to unreplicated DNA.

A second line of evidence linking Ran to the replication checkpoint comes from studies using *Xenopus* egg extracts. Cycling egg extracts mimic early embryonic cell cycles, alternating spontaneously between interphase and mitosis. These extracts arrest the cell cycle if unreplicated DNA is present, which demonstrates that they have an intact S phase checkpoint. A mutant Ran protein (Ran-T24N) that binds tightly to RanGEF and inhibits its activity blocks cycling extracts in interphase (Clarke et al., 1995; Kornbluth et al., 1994). Several observations, including the accumulation of hyperphosphorylated, inactive forms of $p34^{cdk1}$, suggest that this arrest results from activation of the DNA-replication checkpoint. Notably, RanT24N can arrest the cell cycle in this manner even in the absence of DNA, which argues that it activates the DNA-replication checkpoint in a manner that is independent of nucleocytoplasmic transport. Although the data from both *ts*BN2 cells and cycling egg extracts indicate clearly that Ran regulates entry into mitosis, the experimental findings pose one notable problem. In both cases Ran should be driven into its GDP-bound state, through either the loss or inhibition of RanGEF. However, the effects on mitotic progression are opposite, causing loss of the S phase checkpoint in *ts*BN2 cells but inappropriate activation of the checkpoint in egg extracts. This difference might result from differences between the two systems or from the fact that in one case RanGEF is largely absent whereas in the other case it persists, but in an arrested complex.

Although Ran can clearly play some role in the replication checkpoint in metazoans, there is no clear documentation that the S phase checkpoint is disrupted in Ran-pathway mutants in either budding yeast or fission yeast. Matsumoto and Beach (1991) originally reported that mutations in the *S. pombe* RanGEF override the replication checkpoint and cause premature entry into mitosis in the presence of the DNA replication inhibitor hydroxyurea (HU). However, subsequent investigations found that RanGEF mutants in *S. pombe* do not abrogate the S phase checkpoint and that they enter mitosis in the presence of HU only after the cells overcome the arrest and replicate their DNA (Sazer and Nurse, 1994). At the restrictive temperature the RanGEF mutant does not enter mitosis prematurely: it activates the $p34^{cdk1}$-cyclin B complex and enters mitosis with normal timing only after duplicating its

three chromosomes. The chromosomes are then segregated into the two daughter cells, which arrest with hypercondensed chromosomes and a wide medial septum (Sazer and Nurse, 1994). This complex terminal phenotype is probably not due specifically to an accumulation of GDP-Ran, since similar phenotypic consequences arise in fission yeast cells lacking RanGAP, in which GTP-Ran would be expected to accumulate (Matynia et al., 1996). These observations are perhaps more consistent with the notion that the Ran pathway has an important role in the morphological changes associated with mitosis in fission yeast, rather than in the control of the onset of mitosis through the S phase checkpoint.

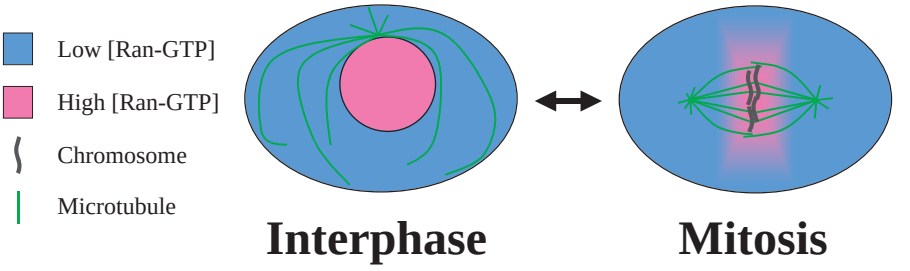
A functional Ran pathway is also required for progression through mitosis. Overexpression of RanBP1 in tissue culture cells or *Xenopus* cycling egg extracts causes arrest in an abnormal mitosis (Battistoni et al., 1997; Kalab et al., 1999). In egg extracts, this mitotic arrest is accompanied by disruption of mitotic spindle assembly (see section IV). It is thus possible that the RanBP1-mediated mitotic arrest is caused by activation of the mitotic spindle assembly checkpoint, although this has not yet been clearly documented.

IV. SPINDLE ASSEMBLY

Several groups have recently demonstrated that disruption of the Ran pathway causes abnormal spindle assembly in *Xenopus* egg extracts (Carazo-Salas et al., 1999; Kalab et al., 1999; Ohba et al., 1999; Wilde and Zheng, 1999). M-phase egg extracts assemble spindles efficiently from demembranated sperm nuclei or chromatinized magnetic beads (Heald et al., 1996; Sawin and Mitchison, 1991). Spindle assembly is severely defective when GTP-Ran levels are lowered in egg extracts: the extracts exhibit low densities of microtubules and disorganized spindle structures. Even more dramatic effects result when GTP-Ran levels are increased, including massive spontaneous assembly of microtubule asters in the absence of chromosomes or centrioles (Kalab et al., 1999; Ohba et al., 1999; Wilde and Zheng, 1999). Remarkably, these asters can fuse into bipolar structures reminiscent of mitotic spindles under some conditions (Kalab et al., 1999). Furthermore, proteins that are normally localized to centrosomes are recruited to these asters, which suggests that microtubule-organizing centers (MTOCs) induced by Ran are highly related in composition to true spindle poles (Ohba et al., 1999; Wilde and Zheng, 1999). These results demonstrate that GTP-Ran can regulate spindle assembly in egg extracts. Moreover, the capacity of Ran to influence spindle dynamics can be clearly distinguished from its function in nucleocytoplasmic transport, because none of these experiments were performed under conditions allowing nuclear envelope assembly at any time.

These reports are consistent with the idea that GTP-Ran regulates tubulin polymerization in a manner that is independent of DNA or centrioles. One straightforward way in which GTP-Ran might promote this dramatic polymerization would be to regulate directly the frequency with which individual microtubules switch between their growing and shrinking states, and favor growth over shrinkage (for a detailed discussion see Desai and Mitchison, 1997). If GTP-Ran does alter the transition of individual microtubules between these states during mitosis, then the localization of RanGEF in mitosis becomes particularly important (Fig. 2). During mitosis the growing plus ends (distal from the centrosome) of microtubules are stabilized near chromosomes through a poorly understood ‘distance effect’, which results from a gradient of a product of an enzymatic reaction associated with chromatin (Tournebize et al., 1997). This stabilization acts by decreasing the frequency with which individual microtubules switch to their shrinking phase (an event termed catastrophe; see Desai and Mitchison, 1997).

During metazoan mitosis, RanGEF associates primarily but not exclusively with chromosomes, and the exchange that presumably occurs there could result in formation of a diffusional GTP-Ran gradient that is inversely proportional to the distance from chromosomes. The association between microtubules and chromatin is quickly lost in extracts treated with RanG19V-GTPγ S, wherein this postulated GTP-Ran gradient would be disrupted (Kalab et al., 1999). The loss of connections between chromosomes and microtubules suggests that the distance effect becomes less important when GTP-Ran levels are high throughout the cytosol, not merely in the vicinity of the chromosomes. Furthermore, RanGEF associates with chromatinized DNA-coated beads, which can therefore recapitulate the polarity of GTP-Ran distribution found near



Nuclear Envelope	Intact	Disassembled
Ran	Nuclear Ran GTP bound Cytosolic Ran GDP bound	[Ran-GTP] inversely proportional to distance from chromosome (?)
Microtubules	$t_{1/2} \approx 5$ minutes	$t_{1/2} \approx 15$ seconds

Fig. 2. Schematic representation of the Ran pathway in metazoan mitosis. During metazoan mitosis Ran is no longer compartmentalized within the nucleus. It is attractive to speculate that Ran-GTP levels are high in the vicinity of chromosomes because of the locally high concentration of chromatin-associated RanGEF. Nuclear envelope breakdown correlates closely with changes in microtubule dynamics from the slow interphase microtubule-turnover rate of around 5 minutes to the rapid mitotic turnover rate of around 15 seconds (Zhai et al., 1996).

mitotic chromosome (Carazo-Salas et al., 1999). These beads can induce spindle assembly in M-phase extracts, but lack this capacity when RanGEF is inhibited, which suggests that establishment of a GTP-Ran gradient plays an essential role in the distance effect.

An alternative target for this activity is the regulation of microtubule-nucleating activities. GTP-Ran is potentially linked to microtubule-nucleating activities by the observation that MTOCs form upon upregulation of GTP-Ran levels, and by the discovery of RanBPM, a centrosomal protein, in a two-hybrid screen for Ran-interacting proteins (Nakamura et al., 1998). In two-hybrid assays, RanBPM binds specifically to a constitutively GTP-bound Ran mutant (RanG19V), but not to a mutant that is likely to be GDP-bound or nucleotide free (RanT24N; Nakamura et al., 1998). The microtubule-nucleating activity of centrosomes and other MTOCs depends upon γ-tubulin ring complexes (γ-TuRC). There are excess, inactive γ-TuRCs in egg extracts (Jeng and Stearns, 1999), but they do not nucleate microtubules until they are recruited to and presumably activated at centrosomes. Overexpression of RanBPM in COS cells caused the reorganization of microtubules and the formation of multiple cytoplasmic γ-tubulin- and RanBPM-containing MTOCs (Nakamura et al., 1998). Furthermore, RanBPM co-fractionates with γ-TuRCs on sucrose gradients, which is consistent with physical association of these proteins, and anti-RanBPM antibodies both inhibit microtubule aster formation on isolated centrosomes in vitro and decrease centrosomal recruitment of γ-tubulin (Nakamura et al., 1998). Future experiments that identify the direct target of the Ran GTPase in spindle formation will undoubtedly determine whether microtubule nucleation or polymerization is the key target of Ran in the regulation of spindle dynamics, and will establish the role of RanBPM in these processes. Indeed, these alternatives are not mutually exclusive, and Ran could regulate both.

Since most experiments directly examining the role of Ran in spindle assembly have been performed in *Xenopus* egg extracts, it is reasonable to ask whether its role in spindle dynamics is a general phenomenon that will be found in other eukaryotes. Some evidence from budding yeast is consistent with this idea. For instance, overexpression of RanGEF suppresses a class of α-tubulin mutations that otherwise display excess nuclear and cytoplasmic microtubules and arrest (Kirkpatrick and Solomon, 1994). Furthermore, overproduction of the *S. cerevisiae* Ran or RanBP1 homologues causes increased rates of chromosome non-disjunction and sensitivity to benomyl, an anti-microtubule drug; this is consistent with the notion that these proteins influence chromosome segregation, although the direct targets of Ran in these processes remains unknown (Ouspenski et al., 1995). Analysis of a particular mutant allele of budding yeast RanBP1 (*yrb1-21*) suggests that this mutation does not significantly disrupt nucleocytoplasmic transport but that it causes substantial defects in progression through mitosis (Ouspenski, 1998). *yrb1-21* cells arrest unbudded with a G_1 DNA content per nucleus, but they frequently become binucleate at the restrictive temperature because they fail to orient the spindle properly within the bud neck of dividing cells. Despite this apparent G_1-phase arrest, their microtubule arrangement is distinct from the aster-like cytoplasmic arrays that are typical of G_1 cells and is more reminiscent of mitotic spindles, although the arrays have a distinct sinusoidal appearance, and microtubules fail to associate within tight bundles (Ouspenski, 1998).

Not all the evidence is consistent with Ran directly regulating spindle assembly in all organisms, however. *ts*BN2 cells can form mitotic spindles at the restrictive temperature when RanGEF activity is lost (Nishitani et al., 1991), which argues against an essential role for Ran in spindle assembly in mammalian cells. Moreover, mutation of RanGEF does not lead to obvious microtubule defects in fission yeast: the cytoplasmic microtubule network is normal, the mitotic spindle forms normally and is capable of separating the duplicated chromosomes, and following mitosis the spindle microtubules are disassembled and cytoplasmic microtubules reform (Matsumoto and Beach, 1993; Sazer and Nurse, 1994). There might, however, be more subtle defects in either microtubule function or attachment of spindle microtubules to abnormally condensed chromatin when the Ran system is malfunctioning. Such defects might explain the observation that diploid fission yeast cells that have only one copy of the gene that encodes Ran lose chromosomes and eventually revert to the haploid state (Matsumoto and Beach, 1993).

Given these different observations, the role of the Ran GTPase system in regulating spindle assembly in vivo in diverse organisms remains uncertain, and systematic examination of this question will be necessary. This is a particularly acute question with regard to yeast, which undergo closed mitosis during which the nuclear envelope remains intact and the mitotic spindle is constructed within the nucleus. In metazoans, it is attractive to suggest that Ran has an informational role regarding the location and/or integrity of the nucleus (Fig. 2): during interphase, cytosolic Ran is GDP bound, whereas nuclear Ran is GTP bound because of chromatin-associated RanGEF, and this asymmetry determines the vectorial nature of nuclear transport. When this gradient is lost during mitosis, nuclear proteins are exposed to GDP-Ran and cytosolic Ran effectors are exposed to GTP-Ran, and this appears to be an important signal initiating spindle assembly. Indeed, the capacity of Ran to work in both of these contexts could allow it to couple NEB with mitotic changes in microtubule dynamics. This would be consistent with observations showing that the shift in microtubule dynamics from the interphase to the mitotic pattern coincides closely with NEB (Zhai et al., 1996).

Nevertheless, the mechanism by which Ran might regulate spindles in yeast would necessarily differ from that proposed above for metazoans, because yeast do not undergo NEB in mitosis. There is no evidence of dissipation of the Ran GTP gradient at mitosis in yeast, and Ran does not re-localize to the cytoplasm at any point in the fission yeast cell cycle (Matynia et al., 1996). In both budding yeast and fission yeast, spindle-microtubule nucleation from the centrosome equivalents (spindle pole bodies) takes place within the nucleus, where the concentration of GTP-Ran is expected to be high. In *S. cerevisiae* GTP-Ran alone could not trigger spindle formation, because the spindle pole body is embedded in the nuclear envelope throughout the entire cell cycle (Byers and Goetsch, 1975). In fission yeast, the spindle pole body is exposed to GTP-Ran at mitotic entry, when the spindle pole body, which lies in the cytoplasm adjacent to the nuclear envelope during interphase, enters the nuclear envelope (Ding et al., 1997). GTP-Ran might

facilitate spindle assembly in yeast, given that assembly occurs within the nucleus, where GTP-Ran levels are high. However, it is difficult to imagine how a gradient of GTP-Ran might be established within nuclei in the absence of RanBP1 and RanGAP-mediated hydrolysis of Ran-bound GTP. Therefore, it will probably be necessary to postulate that the timing of spindle assembly is regulated by events other than modulation of the Ran gradient (e.g. changes in nuclear tubulin concentrations or the nucleating capacity of spindle poles), because there is no clear evidence of gradient variation during the cell cycle.

V. THE ACTIN CYTOSKELETON

Several lines of investigation provide evidence of functional interactions between the Ran system and the actin cytoskeleton in fission yeast. Prior to cell division in *S. pombe*, a medial ring containing actin forms at the center of the cell. This ring contracts after mitosis, and new cell wall material is deposited in its wake, forming a specialized cell wall (the septum) that physically separates the two daughter cells. The normal balance between GTP-Ran and GDP-Ran has been disrupted in *S. pombe* by different strategies, including temperature-sensitive mutations in the RanGEF, overproduction of RanGAP or RanBP1, and deletion of the gene encoding RanGAP or that encoding RanBP1. Under these conditions, actin fails to relocalize properly from the site of septation to the tips of the cells, and the septum becomes abnormally wide. RanGEF also interacts genetically with several previously characterized fission yeast mutants in which septation is defective (J. Demeter and S. Sazer, unpublished results).

Another line of evidence relating the Ran GTPase system to the actin cytoskeleton in fission yeast came from a screen designed to identify high-copy-number suppressors of a temperature-sensitive defect in the RanGEF at its lowest restrictive temperature of 34°C. Whereas screens performed at the higher restrictive temperature of 36°C yielded only RanGEF itself and Ran, this screen was designed to identify Ran targets by finding genes that rescue the defect in the downstream effector pathway(s) that is most sensitive to loss of RanGEF activity (Demeter and Sazer, 1998). One gene discovered in this screen, *imp2*, rescues the viability of the RanGEF mutant at 34°C but not at 36°C. There are proteins with strong structural similarity to Imp2p in budding yeast, fission yeast and mammals, several of which are known to play direct roles in the process of cytokinesis. A GFP-Imp2 fusion protein localizes to a medial ring that contracts during cytokinesis. Overexpression studies in both wild-type and RanGEF temperature-sensitive mutant cells revealed that the biochemical function of Imp2p is to destabilize the actin ring during cytokinesis. This explains the partial rescue of the RanGEF mutant lethality by overexpression of Imp2p: it reduces the percentage of cells that arrest with actin accumulation at the septum. A direct association of Imp2p with components of the Ran GTPase system has not yet been demonstrated.

VI. POST-MITOTIC NUCLEAR ASSEMBLY AND NUCLEAR STRUCTURE

Studies in *Xenopus* egg extracts, *ts*BN2 cells and fission yeast suggest that Ran pathway disruption causes defects in nuclear assembly. When added to *Xenopus* egg extracts, sperm chromatin decondenses and forms functional, replication- and transport-competent nuclei that possess morphologically normal nuclear envelopes. When RanGEF is depleted from egg extracts, sperm chromatin decondenses normally, and a nuclear envelope forms around the chromatin, but the envelope fails to grow to its normal size and DNA replication does not occur (Dasso et al., 1992). Since protein import is also impaired in these experiments, it is not possible to distinguish these defects from a failure of nuclear transport (Dasso et al., 1994). However, nuclear growth and DNA replication are also effectively inhibited after the addition of recombinant RanT24N at concentrations that are apparently insufficient to disrupt protein import (Dasso et al., 1994; Kornbluth et al., 1994). At these low concentrations, RanT24N might block nuclear assembly by decreasing overall RanGEF activity, since recombinant RanGEF can fully restore both nuclear growth and DNA replication. At higher concentrations, RanT24N can inhibit protein import (Hughes et al., 1998). These observations are consistent with the notion that RanT24N must be added in concentrations sufficient to interact with both RanGEF and additional proteins to inhibit transport effectively. Taken together, the observations in *Xenopus* egg extracts not only confirm a role for Ran in nuclear trafficking but also indicate that it has roles in nuclear assembly that are not directly correlated with the overall levels of nuclear transport. The multiple Ran-dependent pathways might be differentially sensitive to functional impairment of the Ran system. However, the loss of DNA replication capacity correlates well with inhibition of nuclear growth, which suggests that it is a secondary consequence of defective nuclear assembly.

Studies in *ts*BN2 cells also link the Ran pathway to nuclear assembly. When shifted to the restrictive temperature during G_1 phase, *ts*BN2 cells do not show gross morphological changes in their nuclear envelopes or nucleoli, but they dramatically re-arrange snRNPs, non-snRNP splicing factors and nuclear poly(A)$^+$ RNAs (Huang et al., 1997). When shifted to the restrictive temperature during S or G_2 phase, *ts*BN2 cells undergo PCC but fail to re-assemble their nuclei properly upon exit from mitosis. Rather, they form several separate micronuclei around their fragmented chromosomes (Nishitani et al., 1991). These micronuclei have envelopes that have an apparently normal morphology but have a reduced volume, which is occupied largely by condensed chromatin (Huang et al., 1997).

Two observations suggest that micronucleation and other *ts*BN2 nuclear defects result from abnormal events during mitosis, rather than a failure of nuclear transport. First, the phenotype of *ts*BN2 cells is closely mimicked by expression of a truncated form of NuMA, a microtubule motor accessory protein that is required for mitotic spindle assembly (Compton and Cleveland, 1993). Furthermore, overexpression of wild-type NuMA partially reverts the micronucleation phenotype of *ts*BN2 cells (Compton and Cleveland, 1993). Although NuMA is a component of the nuclear matrix during interphase, the dispensability of NuMA for nuclear assembly in other contexts argues against increased levels of the protein acting primarily by rescuing some aspect of nuclear architecture (Merdes and Cleveland, 1998; Yang and Snyder, 1992). Second, caffeine causes hydroxyurea-arrested S-phase BHK cells to enter

mitosis with unreplicated DNA in a manner that is very similar to premature chromosome condensation in *ts*BN2 cells (Schlegel and Pardee, 1987). In both cases, the DNA becomes highly condensed as the cells enter mitosis, which results in massive damage to the genome, and post-mitotic nuclear-assembly defects that are also remarkably similar. There is no evidence that caffeine can alter nuclear transport; it is therefore more likely that the common phenotype of *ts*BN2 cells and caffeine-treated BHK cells is a consequence of aberrant chromosome condensation and/or defective spindle assembly and function.

An additional line of evidence relating the Ran GTPase to nuclear envelope structure comes from studies in fission yeast. In *S. pombe*, perturbation of the Ran system leads to a fragmentation of the nuclear envelope after mitosis, despite the fact that the yeast nuclear envelope normally remains intact throughout the entire cell cycle (Demeter et al., 1995; Sazer and Nurse, 1994). Following the progression of synchronous cultures through the cell cycle at the restrictive temperature, it was not possible temporally to separate this nuclear envelope defect from another consequence of the mutation in the RanGEF, failure of chromosome decondensation following mitosis. Therefore, Sazer and co-workers could not determine whether chromosome morphology and nuclear envelope integrity are independently influenced by Ran or whether defects in one are caused by defects in the other (Demeter et al., 1995). However, these observations are consistent with the notion that a functional Ran GTPase system is required for proper post-mitotic nuclear envelope structure in fission yeast. Notably, as in the case of *ts*BN2 cells, gross defects in nuclear morphology are not observed if RanGEF mutants do not pass through mitosis (Demeter et al., 1995), which again indicates that this phenotype is not likely to be the direct result of transport defects but is rather caused by aberrant events occurring during mitosis.

VII. CONCLUSIONS

The past few years have allowed experimental testing of the hypothesis that Ran has a single role, namely in nuclear transport, and that the pleiotropic nature of Ran pathway mutants simply reflects the importance and complexity of nuclear trafficking. In some cases, this notion has held up relatively well, as in the case of mRNA processing and export phenotypes observed in Ran mutants. At this time, we are aware of no evidence that can distinguish disruption of mRNA metabolism in Ran pathway mutants from defects in RNA and protein trafficking. However studies using an in vitro system devoid of nuclei have shown that Ran can directly influence mitotic spindle dynamics. The next challenge will be to demonstrate a similar role for Ran in microtubule dynamics in vivo. Other studies using in vivo and in vitro systems in which nuclei are present have clearly demonstrated the affect of Ran on the onset of mitosis, nuclear assembly and structure, and changes in the actin cytoskeleton. In these cases, it is more difficult to rule out indirect effects of nucleocytoplasmic transport on these processes. However, it is clear that the observed phenotypic consequences of perturbing the Ran system do not correlate with defects in transport. The challenge now will be to understand the role of Ran in these functions with the same biochemical clarity with which we now understand its role in nuclear transport, as well as to understand how these functions are related to each other.

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