Ran-GTP stabilises microtubule asters and inhibits nuclear assembly in *Xenopus* egg extracts

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SUMMARY

Ran is an abundant GTPase of the Ras superfamily that is highly conserved in eukaryotes. In interphase cells, Ran is mainly nuclear and thought to be predominantly GTP-bound, but it is also present in the cytoplasm, probably GDP-bound. This asymmetric distribution plays an important role in directing nucleocytoplasmic transport. Ran has also been implicated in cell cycle control, including the transition from mitosis to interphase when the compartmentalisation of the nucleus is established. Here, we have examined the role of Ran in this transition using a cell-free system of *Xenopus* egg extracts supplemented with sperm heads that provides a model for microtubule aster formation and post-M phase nuclear assembly. Ran-GTP, added as wild-type protein, a mutant defective in GTPase activity (Q69L), or generated by addition of the specific nucleotide exchange factor RCC1, stabilises large microtubule asters nucleated at the sperm centrosome, prevents the redistribution of NuMA from the aster to the nucleus and blocks chromatin decondensation. In contrast, Ran GDP does not stabilise microtubules or inhibit nuclear assembly. RanT24N and RanBP1, which oppose the generation of Ran-GTP by RCC1, arrest nuclear growth after disappearance of the aster. Ran associates with microtubule asters in egg extracts and with mitotic spindles in somatic *Xenopus* cells, suggesting that it may affect microtubule stability directly. These results show that Ran has a novel function in the control of microtubule stability that is clearly distinct from nucleocytoplasmic transport. The Ran GDP/GTP switch may play a role in co-ordinating changes in the structure of microtubules and the assembly of the nucleus associated with the transition from mitosis to interphase.

Key words: Ran, Microtubule, Centrosome, NuMA, Cell cycle control

INTRODUCTION

Ran, a member of the Ras small GTPase superfamily, is highly conserved in eukaryotes from yeast to humans (Rush et al., 1996). Like other GTPases, Ran exists in GTP and GDP bound states that interact differently with regulators and effectors. The major nucleotide exchange factor for Ran, RCC1, is localised in the nucleus where it is thought to generate Ran-GTP (Bischoff and Ponstingl, 1991a; Klebe et al., 1995; Ohtsubo et al., 1989). The intrinsic GTPase activity of Ran is very low, but is stimulated by the interaction of a GTPase-activating protein (RanGAP1) located at the cytoplasmic face of the nuclear pore (Bischoff et al., 1994; Mahajan et al., 1997; Matunis et al., 1996). Ran binding protein 1 (RanBP1) is also predominantly cytoplasmic (Richards et al., 1996) and interacts specifically with the GTP-bound form of Ran (Coutavas et al., 1993), stimulating GTPase activation by RanGAP1 (Bischoff et al., 1995). The compartmentalised localisation of these regulators has been proposed to generate a high concentration of Ran GTP in the nucleus, and a low or very localised concentration of Ran-GTP in the cytoplasm of interphase cells (Görlich and Mattaj, 1996; Görlich et al., 1996). This asymmetric distribution is thought to be crucial for the directionality of transport through the nuclear pore (Görlich et al., 1996; Izaurralde et al., 1997), with RanGTP promoting the assembly of nuclear export complexes in the nucleus which are disassembled by hydrolysis of GTP on Ran in the cytoplasm. Conversely, the assembly of nuclear import complexes in the cytoplasm is promoted by RanGDP and their disassembly after transport into the nucleus is caused by the high concentration of RanGTP there.

The Ran system may also play roles during the cell division cycle, being implicated in changes in nuclear architecture, DNA replication and mitosis (reviewed by Sazer, 1996). Expression of dominant Ran mutants in cultured cells arrests cell cycle progression (Ren et al., 1993, 1994), inhibiting entry into mitosis or progression into S-phase. In a hamster fibroblast cell line that has a temperature sensitive mutation in the RCC1 gene (tsBN2), cells in S-phase undergo chromosome condensation and premature activation of mitotic Cdc2 protein...
kinase activity at the restrictive temperature. Loss of RCC1 also causes the formation of multiple micronuclei and cells fail to pass the G$_1$/S transition (Nishitani et al., 1991). In the fission yeast, *Schizosaccharomyces pombe*, a temperature sensitive mutation of the RCC1 homologue Dcd1/Pim1 produces a defect in exit from mitosis, resulting in condensed chromatin and cell cycle arrest, although Cdc2/cyclin B activity declines (Sazer and Nurse, 1994). Intriguingly, there is fragmentation of the nuclear envelope at the restrictive temperature, even though the nuclear envelope normally remains intact during mitosis in *S. pombe*. This suggests that Dcd1/Pim1 is required for the re-establishment of nuclear organisation following mitosis (Demeter et al., 1995). Nevertheless, it has been difficult to distinguish putative distinct functions for the Ran system from possible secondary effects due to disruption of nucleocytoplasmic transport.

A useful cell-free system to dissect the roles of Ran and its interacting proteins is provided by concentrated cell-free extracts derived from *Xenopus laevis* eggs. Demembranated *Xenopus* sperm heads added to interphase extracts assemble into functional nuclei that undergo DNA replication (Hutchison, 1994). This system provides an in vitro model for the formation of the male pronucleus following fertilisation of the egg and for post-M phase nuclear assembly in general. Previously, we have shown that Ran-GDP is required in this system for the assembly of nuclei competent for DNA replication, whereas Ran-GTP is unable to substitute, possibly indicating a dominant inhibitory effect of Ran-GTP (Hughes et al., 1998). Perturbing the Ran GDP/GTP cycle by adding a mutant of Ran that is defective in GTPase activity (RanQ69L), another mutant does not bind GTP and inhibits RCC1 (RanT24N), or excess Ran BP1, all inhibit nuclear lamina formation, chromatin decondensation and DNA replication, although there are some differences in the gross structure of the chromatin produced (Dasso et al., 1994; Hughes et al., 1998; Kornbluth et al., 1994; Nicolás et al., 1997; Pu and Dasso, 1997).

Here, we show that Ran-GTP, in contrast to RanGDP, arrests pronuclear assembly from sperm chromatin with a large, stable microtubule aster nucleated at the sperm centrosome and blocks chromatin decondensation. Addition of RCC1 produces a similar effect, whereas RanT24N and RanBP1 do not stabilise the asters but arrest nuclear assembly at a later stage. Ran associates with microtubule asters in egg extracts and with the mitotic spindle in cultured cells. This work demonstrates that Ran has a role in the control of microtubule stability that is clearly distinct from nucleocytoplasmic transport and suggests that the Ran-GDP/GTP switch may play an important role in co-ordinating the transition from mitosis to interphase.

**MATERIALS AND METHODS**

**Recombinant proteins**

Recombinant human Ran proteins were produced in *E. coli*, purified and loaded with nucleotides as described previously (Hughes et al., 1998). RanT24N was loaded with GDP, RanQ69L with GTP and wild-type Ran with the nucleotide indicated. *Xenopus* RanBP1 was produced as described by Nicolás et al. (1997). RCC1 (Klebe et al., 1993) was a kind gift from A. Wittinghofer (Dortmund).

**Nuclear assembly and immunofluorescence microscopy**

*Xenopus* egg extracts (LSS) were prepared as described previously (Hutchison, 1994), frozen and stored in aliquots in liquid nitrogen. After thawing, extracts were pre-incubated with added proteins for 60 minutes on ice prior to addition of sperm heads and an ATP regenerating system (Hutchison, 1994). Nuclear assembly and microtubule aster formation was initiated by the addition of demembranated *Xenopus* sperm heads (1,000/ml) followed by incubation at 23°C. To examine nuclear morphology, samples removed at various times during the incubation were fixed and stained on a slide with DAPI (Hutchison, 1994). For immunofluorescence labelling, samples fixed with the EGS (Hutchison, 1994) for 30 minutes at 37°C. The fixed nuclei were then recovered by centrifuging onto coverslips through a cushion of 30% glycerol. To monitor DNA replication, 4 μM biotin-16-DUTP was added to the extracts at the start of the incubation and incorporated biotin was detected with strepavidin conjugated to Texas Red. α-tubulin and NuMA were detected using anti-tubulin antibody (Sigma) and anti-NuMA antibody (Merdes et al., 1996) followed by FITC- and Texas Red-conjugated secondary antibodies (DAKO, Jackson ImmunoResearch Laboratories), respectively (Zhang et al., 1996). Ran was detected with a mouse monoclonal antibody (Transduction Laboratories) raised against residues 7-171 of human Ran, followed by anti-mouse IgG secondary antibody coupled to FITC (DAKO). Images were captured on a Zeiss Axioskop microscope using a cooled CCD camera and processed using Improvision Openlab and Adobe Photoshop software.

**Western blotting**

Proteins were separated on 10% polyacrylamide gels and transferred to nitrocellulose. Blots were blocked with 5% (w/v) milk powder in Tween-Tris buffered saline (TTBS). Ran proteins were detected using the same monoclonal antibody used for immunofluorescence (1:500 dilution in TTBS, overnight at 4°C) followed by HRP-coupled anti-mouse IgG (Bio-Rad) (1:2000 dilution in TTBS, 1 hour at room temperature) and development by ECL (Amersham).

**Xenopus cell culture and indirect fluorescence microscopy**

XTC cells were cultured on coverslips in L-15 medium Leibovitz (Sigma) plus 10% FCS and 25% H$_2$O. The cells were fixed with 4% paraformaldehyde in TBS for 20 minutes on ice and permeabilised with 0.5% Triton X-100 also in TBS for 5 minutes. Indirect fluorescent labelling was carried out by incubating with the first antibody overnight at 4°C followed by incubation with the FITC-conjugated secondary antibody for 60 minutes at room temperature. Images were taken and processed as described above.

**RESULTS**

**Ran-GTP but not Ran-GDP stabilises microtubule asters and inhibits chromatin decondensation**

To investigate the role of the Ran GTpase system in nuclear assembly, we utilised *Xenopus* egg extracts supplemented with demembranated *Xenopus* sperm heads as a model cell-free system. We used interphase extracts containing cyclohexamide which inhibits synthesis of M-phase cyclins and other proteins, permitting passage through the first interphase but preventing subsequent re-entry into M-phase. As shown in Fig. 1, when sperm heads were added to these extracts, microtubules were nucleated at a centrosome formed around the sperm centrioles located at end of each sperm head where the flagellum was present (Félix et al., 1994; Stearns and Kirschner, 1994). By 20 minutes dense asters of elongated microtubules had formed. By 40 minutes, the asters diminished and the condensed...
chromatin rounded up (Fig. 1A). Subsequently, the chromatin decondensed further, a nuclear envelope and lamina were formed, and DNA replication was initiated at 40-60 minutes (data not shown). In this assay, only microtubules specifically nucleated by centrosomes and recovered together with sperm heads by centrifuging onto coverslips were observed, not the randomly nucleated microtubules that form in interphase egg extracts.

When the concentration of Ran-GDP in the extracts (estimated at 1-2 μM; Clarke et al., 1995) was increased by addition of 10 μM recombinant wild-type Ran preloaded with GDP (Fig. 1B), the time course of microtubule aster assembly and disassembly was similar to incubations lacking exogenous Ran (Fig. 1A). RanT24N, which fails to bind GTP, only had a small effect on microtubule stability, slightly delaying aster disassembly (Fig. 1C, 40 minutes). Addition of RanQ69L,

**Fig. 1.** Time course of microtubule aster formation and sperm chromatin decondensation in *Xenopus* egg extracts. Extracts were pre-incubated on ice for 60 minutes with (a) buffer, (b) wild-type Ran-GDP, (c) RanT24N-GDP or (d) RanQ69L-GTP. Ran proteins were added at 10 μM. Demembranated sperm were then added and the extracts incubated for the times shown (in minutes) before fixation, recovery of nuclei by centrifugation onto coverslips, staining for DNA with DAPI and detection of α-tubulin by indirect immunofluorescence.
which is deficient in GTPase activity and is therefore locked in a GTP-bound state, also had no clear effect on microtubule nucleation or the formation of asters. However, this mutant dramatically stabilised the asters during prolonged incubation. In the presence of RanQ69L, the chromatin remained rather elongated and irregularly condensed (Fig. 1D), whereas in extracts supplemented with RanT24N, chromatin became rounded and evenly condensed (Fig. 1C). Although we have previously shown that both of these mutants prevent lamina assembly and DNA replication in this system (Hughes et al., 1998; see also Fig. 5), these results demonstrate that RanQ69L and RanT24N disrupt nuclear assembly at different stages.

Addition of 10 μM wild-type RanGTP also produced large microtubule asters that were stabilised to incubation, very similar to the effect of adding RanQ69L. The chromatin remained elongated and irregularly condensed, indicating that nuclear assembly was inhibited prior to rounding up of the chromatin. The effect of RanQ69L is therefore likely to be due to stabilisation of the GTP-bound state of Ran rather than any other effect of the mutation. No DNA replication occurred in extracts supplemented with Ran-GTP, shown by the lack of incorporation of biotin-dUTP. In contrast, addition of 10 μM exogenous Ran-GDP permitted nuclear assembly and subsequent DNA replication to occur (Fig. 2).

We also perturbed the GDP/GTP state of endogenous Ran without changing the overall concentration by supplementing extracts with regulatory proteins. RCC1, a specific nucleotide exchange factor, generates Ran-GTP from Ran-GDP (Bischoff and Ponstingl, 1991b; Klebe et al., 1995). In contrast, Ran BP1 binds Ran-GTP, opposes the activity of RCC1 and, together with RanGAP1, stimulates the hydrolysis of GTP bound to Ran (Bischoff et al., 1995). Increased concentrations of either RCC1 or RanBP1 inhibit the establishment of DNA replication in this system (Nicolás et al., 1997; Pu and Dasso, 1997). Nevertheless, we found clear differences in their effects on microtubule structure. RanBP1 did not prevent the formation of microtubule asters, or their subsequent disappearance, but produced small nuclei, similar to the effect of adding RanT24N (Fig. 3A). In contrast, RCC1 produced large, stable microtubule asters and irregularly condensed chromatin (Fig. 3B), consistent with the effects of adding Ran-GTP.
RanQ69L affects NuMA localisation

The molecular mechanisms controlling changes in microtubule stability and nuclear assembly at the exit from mitosis are poorly characterised. One protein that has been implicated in post-mitotic nuclear formation is NuMA, a nuclear protein that associates with the mitotic apparatus (Compton and Cleveland, 1993; Gaglio et al., 1995; Merdes and Cleveland, 1998; Merdes et al., 1996). Detection of NuMA with specific antibodies showed a diffuse staining of the microtubule arrays, concentrated around the centrosomal area, as described previously (Merdes et al., 1996). Subsequently, NuMA became concentrated in the forming interphase nuclei (Fig. 4). Small rounded nuclei assembled in the presence of RanT24N also contained NuMA, but in extracts containing RanQ69L, NuMA remained dispersed on the microtubule arrays.

Ran associates with microtubule arrays in Xenopus egg extracts

We determined the localisation of Ran during nuclear assembly by indirect immunofluorescence using a specific antibody that only detects Ran on western blots of Xenopus egg extracts and somatic Xenopus XTC cells (Fig. 5). Ran is absent from demembranated sperm, but rapidly binds to the condensed chromatin after addition to egg extract (Fig. 6A, 5 minutes). After 20 minutes incubation and the assembly of an aster, Ran was found associated with the microtubule array. At 100 minutes, following loss of the aster and nuclear envelope formation, Ran was present in the nucleus (Fig. 6A). When asters were stabilised by RanQ69L (Fig. 6B), Ran was dispersed on the microtubule array and was also associated with the chromatin. With RanT24N, when a small aster was still apparent, Ran was present on the microtubules, but with a more punctate staining than with RanQ69L. Ran also associated with the nucleus in the presence of RanT24N, particularly around the periphery, which probably corresponds to the nuclear envelope (Fig. 6B). With further incubation of extracts containing RanT24N, the aster disappeared, as did the extranuclear Ran staining, whereas

**Fig. 4.** Effect of Ran mutants on microtubule asters and NuMA localisation. RanQ69L arrests extracts with elongated and irregularly condensed chromatin, large microtubule asters and NuMA dispersed on the asters. RanT24N does not stabilise microtubules, but forms nuclei which are small and rounded, with highly condensed chromatin, which contain NuMA. Sperm heads were incubated in egg extracts supplemented with buffer, wild-type Ran-GDP, RanT24N-GDP or RanQ69L-GTP (each at 10 μM) for 100 minutes before processing as in Fig. 2.
Fig. 5. A monoclonal antibody raised against human Ran (residues 7-171) was used to probe a western blot of total proteins from human HeLa cells (lane 1), *Xenopus* XTC cells (lane 2), nuclei assembled in *Xenopus* egg extracts (lane 3) and demembranated *Xenopus* sperm heads (lane 4). The amino acid sequence of *Xenopus* Ran is almost identical to human Ran in this core region (J. Avis and P. R. Clarke, unpublished). The antibody recognises a single polypeptide in XTC cells and assembled nuclei that co-migrates with human Ran at approximately 25 kDa, the predicted molecular mass of Ran. This polypeptide is absent from sperm heads prior to their addition to extracts. On the right are shown the migration positions of molecular mass markers (Bio-Rad): from top to bottom, 103, 76, 49, 33, 28 and 19.9 kDa.

Fig. 6. Ran localises to microtubule asters in egg extracts. (a) Time course of nuclear assembly, showing Ran staining on the condensed chromatin shortly after addition to egg extracts (5 minutes), on microtubule asters (20 minutes) and in the assembled interphase nucleus (100 minutes). (b) Effect of recombinant Ran proteins on Ran localisation. Incubations were performed as in Fig. 3, except that they were carried out for 50 minutes before processing for indirect immunofluorescence of Ran and detection of DNA replication by biotin-dUTP incorporation. Note that the antibody used to detect Ran does not distinguish between the endogenous protein and the added recombinant proteins.
Ran remained on the asters formed in the presence of RanQ69L (Fig. 1C, data not shown).

The localisation of Ran during the cell cycle in Xenopus somatic cells

We also examined the localisation of Ran during the cell cycle in cultured somatic cells (Fig. 7). Previous experiments using mammalian cultured cells have shown that Ran is dispersed throughout the cell during mitosis and is not specifically localised to the mitotic chromosomes (Ren et al., 1993). Using Xenopus laevis XTC cells, we characterised the localisation of Ran in more detail during each stage of mitosis. These cells are particularly useful for this purpose, since they do not round up greatly during mitosis and they allow localisation to specific structures to be seen. In interphase XTC cells, we found that Ran was mainly nuclear, as expected, although there was some weak, dispersed cytoplasmic staining. In prophase, the staining became rather more diffuse throughout the cell, with some concentration at the nuclear envelope. In metaphase, Ran was present throughout the cell, but a sub-population was localised specifically to the mitotic apparatus, particularly the polar/kinetochore microtubules and the spindle poles, but not to the condensed chromosomes. In anaphase, Ran was more concentrated towards the poles of the elongating spindle, but was excluded by the chromosomes. In early telophase, probably before the nuclear envelope is completed, Ran showed a dispersed cytoplasmic staining, and was still clearly excluded from the condensed chromatin. By late telophase, there was a dramatic relocation of most of the Ran to the reassembled daughter nuclei (Fig. 7).

Fig. 7. Localisation of Ran during the cell cycle in Xenopus XTC cells. Cells were grown on coverslips, fixed and processed for indirect immunofluorescence to detect Ran as in Materials and Methods. DNA was stained with DAPI. Selected cells in interphase and at specific stages of mitosis are shown.

Fig. 8. Schematic diagram showing the effect of disrupting the Ran system on nuclear assembly from Xenopus sperm heads. A centrosome at one end of the condensed chromatin is formed around the sperm centrioles at one end of the sperm head following addition to Xenopus egg extract. The centrosome then nucleates a dense aster of microtubules which elongate. The microtubules nucleated by the centrosome subsequently shrink, a nuclear envelope is formed and chromatin rounds up. The nuclear lamina and nuclear matrix are then assembled and DNA replication is initiated. Ran-GTP, either exogenous wild-type or Q69L mutant, or generated by RCC1, stabilises long microtubules and blocks the rounding up and decondensation of the chromatin. In contrast, RanT24N or RanBP1, which disrupt RCC1 activity, inhibit nuclear assembly after completion of the envelope and rounding up of the chromatin, but prior to restarting nucleocytoplasmic transport, nuclear lamina assembly and growth of the nucleus.
The Ran GTPase and its interacting proteins have been implicated in a wide variety of cellular processes (Rush et al., 1996; Sazer, 1996). Of these, only the role of Ran in directing nucleocytoplasmic transport during interphase has been well established. In this study, we have used a cell-free system of *Xenopus* egg extracts to examine the function of Ran during the assembly of pronuclei from demembranated *Xenopus* sperm heads. By characterising changes in microtubule structure and the formation of the nuclear envelope during nuclear assembly, we have shown that disrupting the RanGTPase cycle results in defects at two distinct points (Fig. 8). Increased concentrations of Ran-GTP, produced by addition of recombinant Ran proteins or generated in the extracts by RCC1, produce a large, stable microtubule aster nucleated from the sperm centrosome and arrest nuclear assembly, blocking chromatin decondensation. These effects are clearly distinct from the well characterised role of Ran in nucleocytoplasmic transport.

In contrast to Ran-GTP, excess RanGDP has no inhibitory effects on aster formation or nuclear assembly. This is consistent with the endogenous Ran present in the extracts being predominantly GDP bound, as indicated by interactions with specific partner proteins (Hughes et al., 1998; Nicolás et al., 1997). Disruption of the Ran GTPase cycle with RanT24N or increased concentrations of RanBPI does not stabilise microtubules but arrests nuclear growth at a later stage after rounding up of the chromatin (Fig. 8). Together with previous results (Dasso et al., 1992, 1994; Hughes et al., 1998; Nicolás et al., 1997; Pu and Dasso, 1997), this indicates that the generation of RanGTP by RCC1 specifically inside the nucleus is required for the re-establishment of nucleocytoplasmic transport, the assembly of the nuclear lamina and the initiation of DNA replication. Thus, there may be a switch in the nucleotide state of Ran from GDP to GTP specifically inside the nucleus at exit from mitosis. In somatic cells, where we show that there is a dramatic redistribution of most Ran molecules to daughter nuclei at late telophase when nuclear compartmentalisation is re-established, this would correspond to a change in the predominant form of Ran, although some Ran-GDP would remain in the cytoplasm. In *Xenopus* egg extracts, a large pool of Ran-GDP would remain outside the nuclei.

Since Ran associates with microtubule asters in *Xenopus* egg extracts, it may play a direct role in controlling microtubule dynamics. The localisation of Ran to microtubule asters shows similarities with NuMA, a protein which associates with spindle pole and microtubules during mitosis and relocates to the nucleus in interphase (Compton and Cleveland, 1993; Gaglio et al., 1995; Merdes et al., 1996). Interestingly, truncated NuMA mutants produce multiple micronuclei in hamster cells. This phenotype is strikingly similar to that produced by the loss of RCC1 at the restrictive temperature in hamster tsBN2 cells, conditions where the localisation of NuMA to the micronuclei is defective. Remarkably, overexpression of NuMA can rescue the micronucleation phenotype produced by loss of RCC1 (Compton and Cleveland, 1993). Our results would support the involvement of Ran in the relocation of NuMA from the microtubule aster to the reassembled nucleus. Although NuMA is not required for assembly of interphase nuclei in *Xenopus* egg extracts (Merdes and Cleveland, 1998), blocking its relocalisation or its function might affect microtubule stability. However, the precise role of NuMA in this process and its functional interaction with Ran will require further investigation.

The localisation of Ran to the mitotic apparatus in metaphase cells suggests that Ran, like NuMA (Merdes et al., 1996), may also have a role in formation of the mitotic spindle, although we have not addressed that question here directly. Although the majority of Ran molecules dispersed in the cytoplasm during mitosis are probably GDP-bound, it remains possible that the sub-population of Ran localised to the spindle is GTP-bound. Since RCC1 is localised mainly to the condensed chromosomes during metaphase (C. Zhang and P. R. Clarke, unpublished), a localised concentration of RanGTP could be generated near to the chromosomes and play a role in stabilising the mitotic spindle. At present, we are unable to distinguish the localisation of the GTP- and GDP-bound forms of Ran and test this possibility directly. Recently, a novel, centrosomal protein named RanBP1 has been found to interact preferentially with Ran-GTP in a two-hybrid system of co-expression in yeast (Nakamura et al., 1998). Ran could therefore play a role in microtubule nucleation at the spindle poles during mitosis, although our experiments did not reveal any inhibitory effect of Ran-GTP (or Ran-GDP) on centrosomal nucleation in egg extracts, unlike the effect on microtubule nucleation in tubulin solution reported by Nakamura et al. (1998).

At present, the molecular mechanism by which Ran influences microtubule stability is unknown. Ran could mediate this process through specific interacting proteins (Avis and Clarke, 1996). The functions of such putative effectors could be restricted to mitosis or might also be involved in nucleocytoplasmic transport during interphase. An alternative model is that Ran co-ordinates changes in microtubule structure and the assembly of the interphase nucleus through a single mechanism that is controlled by the GDP/GTP state of Ran. For instance, both microtubule dynamics and nuclear assembly are known to be regulated by protein phosphorylation (Pfüller et al., 1991; Verde et al., 1990). However, in the case of control of these processes by Ran, it is unlikely that stabilisation of mitotic cyclin-dependent protein kinase activity is responsible, since the interphase extracts that we have used are released from the M-phase (CSF) arrested state of the unfertilised eggs during preparation, resulting in degradation of the essential cyclin subunits (Murray, 1991). Furthermore, extracts containing Ran-GTP do not have high histone H1 kinase activity, a measure of mitotic cyclin A- and cyclin B-dependent protein kinases (data not shown). Rather, Ran-GTP may arrest the extracts in a state resembling the transition at the end of mitosis approximately corresponding to the anaphase/telophase boundary when microtubules return to interphase dynamics and the nuclear envelope reforms.

In summary, we have shown that disrupting the RanGTPase cycle results in defects in nuclear assembly at two distinct points. Whereas Ran-GDP is required for nuclear assembly, excess RanGTP stabilises large microtubule asters nucleated by centrosomes and arrests nuclear assembly at an early stage. Inhibition of the generation of RanGTP blocks nuclear growth and initiation of DNA replication probably after completion of the nuclear envelope. These results demonstrate novel effects
of modulating the GDP/GTP state of Ran that are distinct from its interphase function in nucleocytoplasmic transport. They also indicate that the Ran GDP/GTP switch plays a role in coordinating changes in microtubule structure and nuclear assembly at the transition from M-phase to interphase.

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