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COMMENTARY

Ran, a GTPase involved in nuclear processes: its regulators and effectors

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SUMMARY

Ran is a small GTPase that has been implicated in a variety of nuclear processes, including the maintainance of nuclear structure, protein import, mRNA processing and export, and cell cycle regulation. There has been significant progress in determining the role of Ran in nuclear protein import. However, it has been unclear whether this role is sufficient to account for the diverse effects of disrupting Ran functions. Recently, several proteins have been identified that bind specifically to Ran and are, therefore, possible effectors. Other experiments using dominant mutants of Ran that block its GTP/GDP cycle have suggested that Ran may have multiple roles. Here, these results are summarised and discussed with respect to the action of Ran.

Key words: Ran (TC4), GTPase, TC4 (Ran), RCC1

BACKGROUND

Ran (also known as TC4) is an abundant small GTPase of the Ras superfamily, found predominantly in the nucleus of eukaryotic cells (Bischoff and Ponstingl, 1991a,b; Drivas et al., 1990). Homologues of Ran are very highly conserved between species ranging from humans to yeasts and plants. Ran has been implicated in a wide variety of nuclear functions by genetic and biochemical analyses, including chromosome decondensation, assembly of a functional nucleus and initiation of S phase, import of proteins with nuclear localisation signals, pre-mRNA processing, export of proteins and mRNA, and cell cycle coordination. However, its precise molecular function is largely unknown. Like other GTPases, Ran is a molecular switch existing in two states that interact differently with other proteins depending upon whether GTP or GDP is bound (Fig. 1). Determination of the crystal structure of Ran-GDP at 2.3Å resolution has shown a core domain similar to Ras, but with some differences, suggesting that a major conformational change occurs on GTP binding (Scheffzek et al., 1995). The rate of guanine nucleotide exchange on Ran is very low, but an exchange factor, RCC1 (Ohtsubo et al., 1987), specifically stimulates the rate by about 100,000-fold (Klebe et al., 1995). RCC1 is located on chromatin during interphase, although it is not certain that this association is required for its function (Lee et al., 1993; Seino et al., 1992). The loss of functional RCC1 would be expected to result in an inability to generate Ran-GTP, but it also remains possible that RCC1 has an independent function. By analogy with Ras, which acts as a signalling molecule, Ran-GTP might be the ‘active’ form that binds effectors. In this case GTP hydrolysis would switch Ran to the ‘inactive’ GDP-bound state. However, it is apparent that for other Ras-related GTPases, such as the Rab proteins, the GDP-bound form of the protein also interacts with proteins and it is the GTP/GDP cycle that is important for function. The intrinsic rate of GTP hydrolysis by Ran is very low and requires a GTPase activating protein, or RanGAP (Fig. 1). Human RanGAP1 stimulates Ran-GTP hydrolysis by about 100,000-fold (Klebe et al., 1995). Other recent reviews on Ran include those by Rush et al. (1996) and Sazer (1996).

A ROLE FOR RAN IN NUCLEAR TRANSPORT

Fractionation of *Xenopus* oocyte and human somatic cell extracts has identified several components required for the active import of proteins with nuclear localisation sequences (NLS) into nuclei of permeabilised cells (Adam et al., 1990; Moore and Blobel, 1992). One fraction required for import contains importin-α (Görlich et al., 1994) and importin-β (Görlich et al., 1995a), 60 and 95 kDa proteins also known as karyopherin-α and karyopherin-β, respectively (Moroianu et al., 1995). The importins form a heterodimer which complexes with an NLS-containing protein in the cytoplasm (Görlich et al., 1995a; Moore and Blobel, 1993). Importin-α is primarily responsible for NLS recognition, whereas importin-β is involved in docking at the nuclear pore complex (Görlich et al., 1995b; Rexach and Blobel, 1995).

Translocation into the nucleus is energy-dependent and requires a second fraction that contains Ran and a low molecular mass protein p10 (also called pp15/B-2, homologous to NTF2 in yeast) (Melchior et al., 1993a; Moore and Blobel, 1993, 1994b; Paschal and Gerace, 1995). Additional evidence of a role for Ran and its regulators in nucleocytoplasmic transport has come from genetic studies of yeast which have shown that Ran homologues are required for nuclear protein import (Schlenstedt et al., 1995a) and RNA processing and export in *S. cerevisiae* (Kadowaki et al., 1993). These results

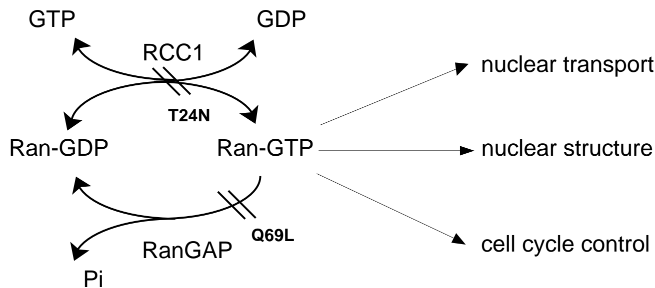

Fig. 1. The Ran GTPase cycle. Guanine nucleotide exchange on Ran is catalysed by RCC1. GTP hydrolysis requires the interaction of a GTPase activating protein (RanGAP). The reactions blocked by the T24N and Q69L mutations of Ran are indicated.

all support the hypothesis that Ran has a fundamental role in nucleocytoplasmic transport which is conserved in eukaryotes as diverse as humans and yeast.

The activity of Ran as a GTPase suggests that its GTP/GDP cycle may be linked to the mechanism of nuclear transport. Indeed, purified recombinant Ran preloaded with a non-hydrolysable GTP analogue blocks protein import (Melchior et al., 1995). The effects of mutant Ran proteins which are disrupted in their GTPase activity have also been studied. Mutation of glutamine 69 to leucine (RanQ69L) or glycine 21 to valine (Ran G21V) results in proteins that are unable to hydrolyse GTP, even when stimulated by RanGAP1, and so are locked in the GTP bound state (Bischoff et al., 1994; Klebe et al., 1995; Ren et al., 1993) (Fig. 1). These GTPase deficient proteins inhibit nuclear protein import in a dominant fashion over the wild-type protein (Schlenstedt et al., 1995a), indicating that nucleocytoplasmic transport requires GTP hydrolysis on Ran. It has been proposed that GTP bound to Ran is hydrolysed at the nuclear pore complex upon docking of transport substrates (Melchior et al., 1995) or during translocation through the pore complex (Moore and Blobel, 1994a). Evidence for a direct interaction between Ran and the importin/NLS-ligand complex was provided by Rexach and Blobel (1995), who found that, using purified recombinant yeast proteins, Ran-GTP disrupts the complex between the karyopherins/importins and an NLS protein via an interaction with importin-β. These authors suggested that the Ran GTP/GDP cycle may be linked to the recycling of importin-β, permitting the assembly of a new importin/NLS protein complexes. More recently, Nehrbass and Blobel (1996), also using a reconstituted assembly reaction, have found that the yeast p10/NTF2 transport factor binds Ran GDP but not Ran GTP, also binds importin-β and promotes the assembly of the pentmeric complex with importin-α and a nucleoporin. Addition of GTP to the complex promoted the dissociation of importin-α. Previously, it had not been clear how Ran-GTP required for transport was generated in the cytoplasm, since RCC1 is nuclear. These authors suggested that a nucleotide exchange reaction occurs in the complex, forming Ran GTP in situ (Moore, 1996; Nehrbass and Blobel, 1996). Human RanGAP1 is homologous in sequence to the product of RNA1 in yeast, which seems to be cytosolic or located at the nuclear envelope (Becker et al., 1995; Bischoff et al., 1994, 1995a; Hooper et al., 1990; Melchior et al., 1993b). Nehrbass and Blobel (1996) suggest that the function of cytoplasmic RanGAP may be to prevent the formation of Ran GTP which would inhibit importin complex formation. In this model, Ran-GDP initially forms a transient complex with the importins when docking at the nuclear pore, then nucleotide exchange results in dissociation and movement of the transport substrate bound to importin-α through the pore. However, this model does not yet account for all of the sequence of events during the translocation of proteins through the pore complex; what happens to the components when the transient complex dissociates and when does GTP hydrolysis occur? The role of proteins present in the nuclear pore complex that specifically bind Ran-GTP (see below) is also not clear. The activity of RCC1 would not be necessary for nuclear import in this model, although inactivation of mammalian RCC1 or its yeast homologue PRP20 causes suppression of protein import, as well as defects in RNA processing and export (Cheng et al., 1995; Kadowaki et al., 1993; Tachibana et al., 1994). RCC1 might be required for the generation of Ran-GTP within the nucleus for intranuclear transport, export from the nucleus (Moroianu and Blobel, 1995) or of the recycling nuclear components.

NUCLEAR STRUCTURE

RCC1 was first identified in a temperature sensitive hamster cell line (tsBN2) that has a point mutation in the RCC1 gene which results in instability of the encoded protein at the restrictive temperature. These cells show a number of defects when the temperature is shifted: cells in G_1 fail to enter S-phase, while cells already in S-phase display premature activation of the Cdc2/cyclin B protein kinase that regulates entry into mitosis and chromosome condensation (Nishitani et al., 1991). In the fission yeast, *Schizosaccharomyces pombe*, a temperature sensitive mutant of an RCC1 homologue, the product of the *pim1*/*dcd* gene, enters mitosis on schedule, but fails to undergo chromatin decondensation and subsequent DNA replication following mitosis, although Cdc2/cyclin B protein kinase activity declines as normal (Sazer and Nurse, 1994). In addition, the nuclear envelope fragments in these mutants, even though it normally remains intact during the cell cycle in *S. pombe* (Demeter et al., 1995). Perhaps this defect is closely related to structural changes seen in the nuclei of tsBN2 cells when RCC1 is lost. However, it seems that in *S. pombe*, and budding yeast, the cell cycle aberations of tsBN2 cells are not immediately apparent upon the loss of RCC1 homologues.

The role of Ran and RCC1 in chromatin decondensation and nuclear assembly has also been investigated in interphasic *Xenopus* egg extracts. Condensed chromatin added to these extracts undergoes a process of decondensation and accumulation of membrane vesicles that form a nuclear envelope, followed by the import of proteins, the swelling of the nucleus and the initiation of DNA replication. Prior depletion of RCC1 using precipitating antibodies results in small nuclei with condensed chromatin that fail to initiate DNA replication (Dasso et al., 1992). A dominant inactive mutant of Ran which is unable to bind GTP and forms a stable complex with RCC1, RanT24N, produces a similar effect (Kornbluth et al., 1994), which can be overcome by prior addition of an excess of RCC1

(Dasso et al., 1994). The small nuclei formed in the presence of RanT24N are unable to undergo DNA replication, but may continue to import proteins and have an intact nuclear envelope (Kornbluth et al., 1994) (P. R. Clarke et al., unpublished). Whether the requirement for RCC1 shown by these experiments is due solely to its activity as a nucleotide exchange factor for Ran or whether RCC1 has an independent role is unclear, although it has been suggested that Ran-GTP can overcome at least some of the effects of removing RCC1 (Dasso et al., 1994). Interestingly, dominant mutants of Ran that probably inhibit nuclear transport do not seem to prevent chromatin decondensation (Kornbluth et al., 1994) (P. R. Clarke et al., unpublished), indicating that RCC1 and/or Ran play a role in the maintainance of interphasic nuclear structure that is distinct from effects on nucleocytoplasmic transport. One possibility is that RCC1 interacts with additional proteins that carry out a role in maintaining the structure of the nucleus and this function is blocked by the stable association of Ran T24N.

CELL CYCLE CONTROL

The premature mitotic phenotype of the tsBN2 cell line at the restrictive temperature led to the suggestion that RCC1 is part of a cell cycle checkpoint mechanism (Nishitani et al., 1991). RCC1, located on the chromatin would detect the progression of S-phase and signal through Ran to regulate activation of Cdc2/cyclin B protein kinase and control entry into mitosis. Other evidence for a role of Ran in the control of cell cycle progression has come from experiments in which mutants of Ran defective in GTPase activity produced cell cycle arrests prior to entry into S-phase and at the G_2/M transition when transfected into human cells (Ren et al., 1993, 1994). One potential difficulty with the interpretation of these experiments, however, is that the disruption of the RanGTP/GDP cycle may also affect nucleocytoplasmic transport and/or nuclear structure, which could perturb cell cycle progression or trigger cell cycle checkpoints indirectly. More direct evidence for a role of Ran in the regulation of Cdc2/cyclin B has come from a cell-free system of *Xenopus* egg extracts, where RanT24N inhibits kinase activation, indicating that RCC1 and/or Ran-GTP is required for this process (Clarke et al., 1995; Kornbluth et al., 1994). Together, these experiments suggest the reverse of the initial hypothesis that Ran-GTP would inhibit Cdc2/cyclin B activation (Nishitani et al., 1991; Ren et al., 1994). Furthermore, the RanQ69L mutant which is locked in the GTP-bound form does not block Cdc2/cyclin B activation in *Xenopus* egg extracts (Clarke et al., 1995). The effect of Ran T24N does not appear to be an indirect one due to inhibition of chromatin decondensation, DNA replication or nuclear protein import (Clarke et al., 1995; Kornbluth et al., 1994). However, it remains possible that the interaction of Ran T24N with the nucleus triggers a cell cycle checkpoint, since the presence of nuclei greatly potentiates the ability of Ran T24N to inhibit Cdc2/cyclin B activation (Clarke et al., 1995). Nevertheless, for Ran to have a controlling influence on the progression of the cell cycle, it might be expected that the RanGTP/GDP ratio would change during phase transitions or when cell cycle checkpoints are activated. Evidence for this is currently lacking.

THE SEARCH FOR DOWNSTREAM EFFECTORS OF RAN

In order to understand the role of Ran in these various processes, it would clearly be useful to identify and characterise the putative effectors with which it interacts. The first such protein to be identified has been named Ran-binding protein 1 (RanBP1), a 24 kDa polypeptide in mammalian cells that interacts specifically with Ran-GTP (Coutavas et al., 1993). This interaction requires a short stretch of acidic residues (DEDDDL) at the carboxy terminus of Ran (Ren et al., 1995). In experiments using purified proteins, RanBP1 inhibits GTP dissociation catalysed by RCC1 and promotes the activity of RanGAP (Bischoff et al., 1995b; Hayashi et al., 1995). RanBP1 can also form a trimeric complex with RCC1 and Ran in the nucleotide-free transition state (Bischoff et al., 1995b). One possibility is that RanBP1 is not a true effector of Ran but rather a regulator of its GTP/GDP cycle. In budding yeast, the 34 kDa protein Yrp1p (also called CST20), is about 40% identical to murine RanBP1, although it has a short extension at the amino terminus and is missing a highly charged sequence at the carboxy terminus of murine RanBP1. Mammalian Ran BP1 also contains a potential RNA-binding motif that is absent in yeast Yrp1p, but both have potential leucine zipper regions (Ouspenski et al., 1995; Schlenstedt et al., 1995b) (Fig. 2). As predicted, Yrb1p interacts specifically with Ran-GTP; it is not yet clear if Yrb1p is a functional homologue of mammalian RanBP1. Loss of function mutants of the YRB1 gene in yeast are defective in nuclear transport (Schlenstedt et al., 1995b). Recently, Lounsbury et al. (1996) have provided evidence that association with Ran BP1 increases the affinity of Ran-GTP for importin-β, suggesting a role for Ran-binding domains in stabilising protein import complexes.

Further Ran-binding proteins have been identified that have domains homologous to RanBP1 (Fig. 2). The best characterised of these has been discovered independently by two groups. Yokoyama et al. (1995) used the two hybrid screen to look for interaction with Ran in yeast and constructed an open reading frame from overlapping clones which encodes a protein of predicted molecular mass 358 kDa. Wu et al. (1995) achieved the same result by expression cloning using Ran-GTP as the bait. The protein, named Ran binding protein 2 (RanBP2) or Nup358, locates at the nuclear pore, near the tips of the cytoplasmically exposed fibres and antibodies directed against RanBP2 will inhibit nuclear import activity in a cell-free assay (Yokoyama et al., 1995). Intriguingly, RanBP2 contains four domains homologous to RanBP1 which can each interact with Ran-GTP, although with differing affinities (Yokoyama et al., 1995). Within the amino acid sequence are also FG and XFXFG peptide repeats, signature motifs of a series of nucleoporins. Ran BP2 could therefore also interact with protein import complexes via importin-β or p10 binding to these regions and it may be the site of docking at the pore complex. In addition, there is a large leucine rich domain in the amino-terminal of Ran BP2 that may be involved in protein-protein interactions, eight zinc finger motifs similar to those found in Nup153, and a carboxy-terminal domain with homology to cyclophilin A. The functions of these domains are unknown at present.

Beddow et al. (1995) have described the expression cloning

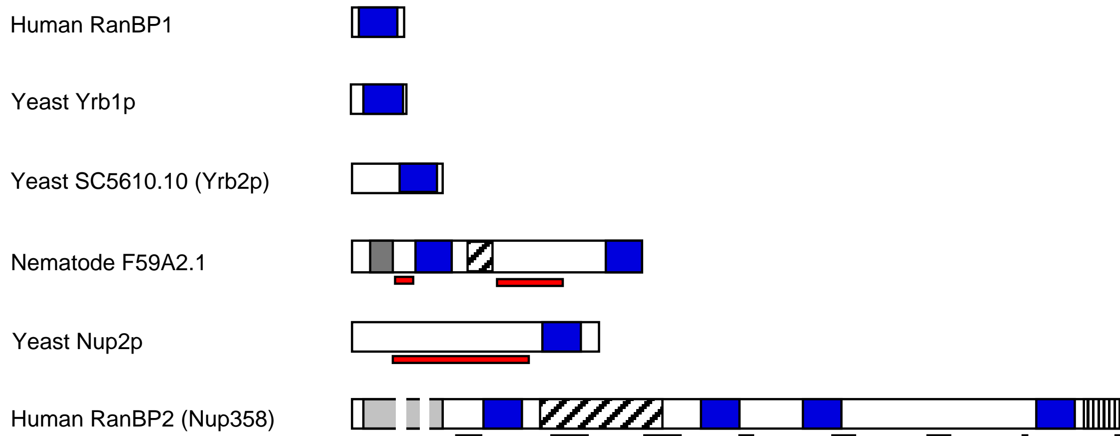

Fig. 2. Domain organisation of proteins containing the Ran binding domain (RBD). Blue filled boxes represent the RBD; the hatched boxes indicate Nup153-like zinc-finger domains; the dotted box represents a Leucine-rich domain; the grey box is a glutamine-rich region 40-50% identical to regions found in TFIID and galII (a QA domain), and the striped box represents a cyclophilin-homologous domain. Red horizontal bars represent regions containing XFXFG and FG repeats characteristic of some nucleoporins. The breaks in the leucine-rich domain of human RanBP2 mean that this region is actually larger than represented in the diagram.

of two proteins of 50 and 96 kDa, respectively, using Ran-[^{32}P]GTP to probe a human hippocampus library. These proteins also possess regions showing a high degree of identity with RanBP1, and analysis of the binding activity of protein fragments enabled the authors to define a Ran-binding domain (RBD), represented by a highly conserved sequence motif of ~150 residues, also noted by others (Dingwall et al., 1995; Hartmann and Görlich, 1995). Searches of the GenBank database for sequences containing the RBD have yielded the budding yeast sfo1 gene and a genomic sequence from *C. elegans*; F59A2.1. The overall domain structure for F59A2.1 is shown in Fig. 2. It has a QA-rich N-terminal domain found in some transcription factors, copies of the FG and XFXFG sequence repeats characteristic of nuclear pore proteins (nucleoporins), and two RBDs. Sequence similarity has also been noted with regions in a putative protein identified in the yeast genome (SC5610.10), and a known yeast nucleoporin, Nup2p. The single putative RBD in Nup2p is at the C terminus and the isolated region of the protein can interact with yeast Ran in a two-hybrid system (Dingwall et al., 1995), although the significance of this interaction is unclear. The roles of these potential effectors of Ran remain to be determined.

CONCLUSIONS

Already much has been learnt about the cellular functions of Ran, its regulators and its putative effectors. However, the relationships between their roles in nuclear transport, nuclear structure and regulation of the cell cycle are still unclear. The question remains of whether Ran has a single primary function, or several distinct functions. The best characterised role for Ran at present is in nucleocytoplasmic transport. However, this role does not seem to be able to account for all of the effects of perturbing the Ran GTP/GDP cycle upon nuclear structure and cell cycle progression. To determine precisely how Ran works, we need to know more about its interacting proteins and their biochemical functions. Regulators that have been identified are GTPase activating proteins and RCC1, a nucleotide exchange factor. Several putative effector proteins containing one or more sequences corresponding to a conserved Ran-binding domain have been found, some of which are probably located at the nuclear pore providing a link to nuclear transport. Other proteins that associate with Ran, such as p10 and importin-β, involved in nuclear protein import, do not contain this motif. Additional interacting proteins playing other roles may still await discovery.

REFERENCES

Adam, S. A., Sterne-Marr, R. and Gerace, L. (1990). Nuclear import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J. Cell Biol.* **111**, 807-816.

Becker, J., Melchior, F., Gerke, V., Bischoff, F. R., Ponstingl, H. and Wittinghofer, A. (1995). RNA1 encodes a GTPase-activating protein specific for Gsp1p, the Ran/TC4 homologue of Saccharomyces cerevisiae. *J. Biol. Chem.* **270**, 11860-11865.

Beddow, A. L., Richards, S. A., Orem, N. R. and Macara, I. G. (1995). The Ran/TC4 GTPase-binding domain: identification by expression cloning and characterization of a conserved sequence motif. *Proc. Nat. Acad. Sci. USA* **92**, 3328-3332.

Bischoff, F. R. and Ponstingl, H. (1991a). Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. *Nature* **354**, 80-82.

Bischoff, F. R. and Ponstingl, H. (1991b). Mitotic regulator protein RCC1 is complexed with a nuclear ras-related polypeptide. *Proc. Nat. Acad. Sci. USA* **88**, 10830-10834.

Bischoff, F. R., Klebe, C., Kretschmer, J., Wittinghofer, A. and Ponstingl, H. (1994). RanGAP1 induces GTPase activity of nuclear Ras-related Ran. *Proc. Nat. Acad. Sci. USA* **91**, 2587-2591.

Bischoff, F. R., Krebber, H., Kempf, T., Hermes, I. and Ponstingl, H. (1995a). Human RanGTPase-activating protein RanGAP1 is a homologue of yeast Rna1p involved in mRNA processing and transport. *Proc. Nat. Acad. Sci. USA* **92**, 1749-1753.

Bischoff, F. R., Krebber, H., Smirnova, E., Dong, W. and Ponstingl, H. (1995b). Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. *EMBO J.* **14**, 705-715.

Cheng, Y., Dahlberg, J. E. and Lund, E. (1995). Diverse effects of the guanine nucleotide exchange factor RCC1 on RNA transport. *Science* **267**, 1807-1810.

Clarke, P. R., Klebe, C., Wittinghofer, A. and Karsenti, E. (1995). Regulation of cdc2/cyclin B activation by Ran, a Ras-related GTPase. *J. Cell Sci.* **108**, 1217-1225.

Coutavas, E., Ren, M., Oppenheim, J. D., D'Eustachio, P. and Rush, M. G.

(1993). Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. *Nature* **366**, 585-587.

Dasso, M., Nishitani, H., Kornbluth, S., Nishimoto, T. and Newport, J. W. (1992). RCC1, a regulator of mitosis, is essential for DNA replication. *Mol. Cell. Biol.* **12**, 3337-3345.

Dasso, M., Seki, T., Azuma, Y., Ohba, T. and Nishimoto, T. (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.

Demeter, J., Morphew, M. and Sazer, S. (1995). A mutation in the RCC1-related protein pim1 results in nuclear envelope fragmentation in fission yeast. *Proc. Nat. Acad. Sci USA* **92**, 1436-1440.

Dingwall, C., Kandels-Lewis, S. and Séraphin, B. (1995). A family of Ran binding proteins that includes nucleoporins. *Proc. Nat. Acad. Sci. USA* **92**, 7525-7529.

Drivas, G. T., Shih, A., Coutavas, E., Rush, M. G. and D'Eustachio, P. (1990). Characterization of four novel ras-like genes expressed in a human teratocarcinoma cell line. *Mol. Cell. Biol.* **10**, 1793-1798.

Görlich, D., Prehn, S., Laskey, R. A. and Hartmann, E. (1994). Isolation of a protein that is essential for the first step of nuclear protein import. *Cell* **79**, 767-778.

Görlich, D., Kostka, S., Kraft, R., Dingwall, C., Laskey, R., Hartmann, E. and Prehn, S. (1995a). Two different subunits of importin cooperate to recognise nuclear localisation signals and bind them to the nuclear envelope. *Curr. Biol.* **5**, 383-392.

Görlich, D., Vogel, F., Mills, A. D., Hartmann, E. and Laskey, R. A. (1995b). Distinct functions for the two importin subunits in nuclear protein import. *Nature* **377**, 246-248.

Hartmann, E. and Görlich, D. (1995). A Ran-binding motif in nuclear pore proteins. *Trends Cell Biol.* **5**, 192-193.

Hayashi, N., Yokoyama, N., Seki, T., Azuma, Y., Ohba, T. and Nishimoto, T. (1995). RanBP1, a Ras-like nuclear G protein binding to Ran/TC4, inhibits RCC1 via Ran/TC4. *Mol. Gen. Genet.* **247**, 661-669.

Hooper, A., Tragila, H. M. and Dunst, R. W. (1990). The yeast RNA1 gene product necessary for RNA processing is located in the cytosol and apparently excluded from the nucleus. *J. Cell Biol.* **111**, 309-321.

Kadowaki, T., Goldfarb, D., Spitz, L. M., Tartakoff, A. M. and Ohno, M. (1993). Regulation of RNA processing and transport by a nuclear guanine nucleotide release protein and members of the Ras superfamily. *EMBO J.* **12**, 2929-2937.

Klebe, C., Bischoff, F. R., Ponstingl, H. and Wittinghofer, A. (1995). Interaction of the nuclear GTP-binding protein Ran with its regulatory proteins RCC1 and RanGAP1. *Biochemistry* **34**, 639-647.

Kornbluth, S., Dasso, M. and Newport, J. (1994). Evidence for a dual role for TC4 protein in regulating nuclear structure and cell cycle progression. *J. Cell Biol.* **125**, 705-719.

Lee, A., Tam, R., Belhumeur, P., DiPaolo, T. and Clark, M. W. (1993). Prp20, the Saccharomyces cerevisiae homolog of the regulator of chromosome condensation, RCC1, interacts with double-stranded DNA through a multi-component complex containing GTP-binding proteins. *J. Cell Sci.* **106**, 287-298.

Lounsbury, K. M., Richards, S. A., Perlungher, R. R. and Macara, I. G. (1996). Ran binding domains promote the interaction of Ran with p97/b-karyopherin, linking the docking and translocation steps of nuclear import. *J. Biol. Chem.* **271**, 2357-2360.

Melchior, F., Paschal, B., Evans, J. and Gerace, L. (1993a). Inhibition of nuclear protein import by nonhydrolyzable analogues of GTP and identification of the small GTPase Ran/TC4 as an essential transport factor. *J. Cell Biol.* **123**, 1649-1659.

Melchior, F., Weber, K. and Gerke, V. (1993b). A functional homologue of the RNA1 gene product in Schizosaccharomyces pombe: purification, biochemical characterisation, and identification of a leucine-rich repeat motif. *Mol. Biol. Cell* **4**, 569-581.

Melchior, F., Guan, T., Yokoyama, N., Nishimoto, T. and Gerace, L. (1995). GTP hydrolysis by Ran occurs at the nuclear pore complex in an early step of protein import. *J. Biol. Chem.* **131**, 571-581.

Moore, M. S. and Blobel, G. (1992). The two steps of nuclear import, targeting to the nuclear envelope and translocation through the nuclear pore, require different cytosolic factors. *Cell* **69**, 939-950.

Moore, M. S. and Blobel, G. (1993). The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. *Nature* **365**, 661-663.

Moore, M. S. and Blobel, G. (1994a). A G protein involved in nucleocytoplasmic transport: the role of Ran. *Trends Biochem. Sci.* **19**, 211-216.

Moore, M. S. and Blobel, G. (1994b). Purification of a Ran-interacting protein that is required for protein import into the nucleus. *Proc. Nat. Acad. Sci. USA* **91**, 10212-10216.

Moore, M. S. (1996). Generation of GTP-Ran for nuclear protein import. *Science* **272**, 47.

Moroianu, J. and Blobel, G. (1995). Protein export from the nucleus requires the GTPase Ran and GTP hydrolysis. *Proc. Nat. Acad. Sci. USA* **92**, 4318-4322.

Moroianu, J., Blobel, G. and Radu, A. (1995). Previously identified protein of uncertain function is karyopherin α and together with karyopherin β docks import substrate at the nuclear pore complex. *Proc. Nat. Acad. Sci. USA* **92**, 2008-2011.

Nehrbass, U. and Blobel, G. (1996). Role of the nuclear transport factor p10 in nuclear transport. *Science* **272**, 120-122.

Nishitani, H., Ohtsubo, M., Yamashita, K., Iida, H., Pines, J., Yasudo, H., Shibata, Y., Hunter, T. and Nishimoto, T. (1991). Loss of RCC1, a nuclear DNA-binding protein, uncouples the completion of DNA replication from the activation of cdc2 protein kinase and mitosis. *EMBO J.* **10**, 1555-1564.

Ohtsubo, M., Kai, R., Furuno, N., Sekiguchi, T., Sekiguchi, M., Hayashida, H., Kuma, K., Miyata, T., Fukushige, S., Murotsu, T., Matsubara, K. and Nishimoto, T. (1987). Isolation and characterisation of the active cDNA of the human cell cycle gene (RCC1) involved in the regulation of onset of chromosome condensation. *Genes Dev.* **1**, 585-593.

Ouspenski, I. I., Mueller, U. W., Matynia, A., Sazer, S., Elledge, S. J. and Brinkley, B. R. (1995). Ran-binding protein-1 is an essential component of the Ran/RCC1 molecular switch system in budding yeast. *J. Biol. Chem.* **270**, 1975-1978.

Paschal, B. M. and Gerace, L. (1995). Identification of NTF2, a cytosolic factor for nuclear import that interacts with nuclear pore complex protein p62. *J. Cell Biol.* **129**, 925-937.

Ren, M., Drivas, G., D'Eustachio, P. and Rush, M. G. (1993). Ran/TC4: a small nuclear GTP-binding protein that regulates DNA synthesis. *J. Cell Biol.* **120**, 313-323.

Ren, M., Coutavas, E., D'Eustachio, P. and Rush, M. G. (1994). Effects of mutant Ran/TC4 proteins on cell cycle progression. *Mol. Cell Biol.* **14**, 4216-4224.

Ren, M., Villamarin, A., Shih, A., Coutavas, E., Moore, M. S., LoCurcio, M., Clarke, V., Oppenheim, J. D., D'Eustachio, P. and Rush, M. G. (1995). Separate domains of the Ran GTPase interact with different factors to regulate nuclear protein import and RNA processing. *Mol. Cell Biol.* **15**, 2117-2124.

Rexach, R. and Blobel, G. (1995). Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell* **83**, 683-692.

Rush, M. G., Drivas, G. and D'Eustachio, P. D. (1996). The small nuclear GTPase Ran: how much does it run? *BioEssays* **18**, 103-112.

Sazer, S. and Nurse, P. (1994). A fission yeast RCC1-related protein is required for the mitosis to interphase transition. *EMBO J.* **13**, 606-615.

Sazer, S. (1996). The search for the primary function of the Ran GTPase continues. *Trends Cell Biol.* **6**, 81-85.

Scheffzek, K., Klebe, C., Fritz-Wolf, K., Kabsch, W. and Wittinghofer, A. (1995). Crystal structure of the nuclear Ras-related protein Ran in its GDP-bound form. *Nature* **374**, 378-381.

Schlenstedt, G., Saavedra, C., Loeb, J. D., Cole, C. N. and Silver, P. A. (1995a). The GTP-bound form of the yeast Ran/TC4 homologue blocks nuclear protein import and appearance of poly(A)$^{+}$ RNA in the cytoplasm. *Proc. Nat. Acad. Sci. USA* **92**, 225-229.

Schlenstedt, G., Wong, D. H., Koepp, D. M. and Silver, P. A. (1995b). Mutants in a yeast Ran binding protein are defective in nuclear transport. *EMBO J.* **14**, 5367-5378.

Seino, H., Hisamoto, N., Uzawa, S., Sekiguchi, T. and Nishimoto, T. (1992). DNA-binding domain of RCC1 protein is not essential for coupling mitosis with DNA replication. *J. Cell Sci.* **102**, 393-400.

Tachibana, T., Imamoto, N., Seino, H., Nishimoto, T. and Yoneda, Y. (1994). Loss of RCC1 leads to suppression of nuclear protein import in living cells. *J. Biol. Chem.* **269**, 24542-24545.

Wu, J., Matunis, M. J., Kraemer, D., Blobel, G. and Coutavas, E. (1995). Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. *J. Biol. Chem.* **270**, 14209-14213.

Yokoyama, N., Hayashi, N., Seki, T., Pante, N., Ohba, T., Nishii, K., Kuma, K., Hayashida, T., M iyata, T., Aebi, U., Fukui, M. and Nishimoto, T. (1995). A giant nucleopore protein that binds Ran/TC4. *Nature* **376**, 184-188.