Targeting of Ran: variation on a common theme?

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Summary

The Ran GTPase plays a key role in nucleocytoplasmic transport. In its GTP-bound form, it directly interacts with members of the importin β family of nuclear transport receptors and modulates their association with cargo. Work in cell-free higher-eukaryote systems has demonstrated additional roles for Ran in spindle and nuclear envelope formation during mitosis. However, until recently, no Ran-target proteins in these cellular processes were known. Several groups have now identified importin β as one important target of Ran during mitotic spindle formation. This finding suggests that Ran uses the same effectors to regulate different cellular processes.

Introduction

Small GTPases of the Ras superfamily regulate many cellular processes, including growth, morphogenesis, cell motility, axonal guidance, cytokinesis, and trafficking through the Golgi, endosomes and nucleus (Takai et al., 2001). Because of their low intrinsic GTP hydrolysis and guanine-nucleotide-exchange activity, these proteins exist in two states, GTP bound and GDP bound, whose interconversion is regulated by GTPase-activating proteins (GAPs), guanine-nucleotide exchange factors (GEFs) and GDP-dissociation inhibitors (GDIs). The GTPases act as molecular switches, in which the ‘on’ or ‘active’ state is the GTP-bound form. The GTP-bound state interacts with targets or effectors that link the switch to specific cellular processes.

The small Ras-like GTPase Ran, one of the most highly conserved proteins in eukaryotes, is essential for cell viability in all organisms tested (Macara et al., 2000). Alterations in the Ran GTPase cycle produce a very pleiotropic phenotype. Whether this reflects functions of Ran in multiple cellular processes or merely its pivotal role in nucleocytoplasmic transport has been debated for some time. The recent demonstration that Ran functions in mitotic spindle and nuclear envelope formation independently of its role in nucleocytoplasmic transport (Hetzer et al., 2000; Sazer and Dasso, 2000; Zhang and Clarke, 2000; Zhang andClarke, 2001) argues for the former explanation. However, in contrast to the role of Ran in nucleocytoplasmic transport, the molecular mechanisms of these additional functions of Ran are poorly understood. Here, we give an overview of the proteins in higher and lower eukaryotes that are known to bind to the two nucleotide-bound states of Ran. We hypothesize that the importin-β-like family of nuclear transport receptors represents, in a strict sense, the only Ran target known to date. Interestingly, recent evidence demonstrates that this family of proteins at least partially underpins the function of Ran in mitotic spindle formation as well as its crucial role in nucleocytoplasmic transport. This suggests that the same class of proteins can link a given GTPase to different cellular processes (Dasso, 2001; Melchior, 2001).

The Ran GTPase cycle

In contrast to other members of the Ras GTPase superfamily, Ran is a very abundant, soluble and predominantly nuclear protein (Macara et al., 2000). A crucial feature of the Ran GTPase cycle is the spatial separation of GTP hydrolysis and guanine-nucleotide exchange by the nuclear envelope (NE). This separation is a consequence of the different subcellular localizations of the known Ran-specific GAP and GEF. The only known RanGAP, RanGAP1 in higher eukaryotes or Rna1p in yeast, is a crescent-shape protein, formed by eleven leucine-rich repeats, that bears no resemblance to RasGAP or RhoGAP (Hillig et al., 1999) and localizes exclusively to the cytosol. However, a pool of RanGAP1 is associated with Nup358/RanBP2, a component of the cytoplasmic filaments of the mammalian nuclear pore complex (NPC; Mahajan et al., 1998; Matunis et al., 1998; Saitoh et al., 1998). This association depends on the post-translational modification of RanGAP1 by a ubiquitin-related protein, SUMO-1. Rna1p lacks the domain of RanGAP1 that is modified and there is no evidence for a modification of this kind. However, there is evidence that Rna1p is associated with NPCs (Allen et al., 2001).

Although the association of RanGAP1 with RanBP2 appears to be important for nucleocytoplasmic transport in some permeabilized cell systems, the facts that yeast and flies do not contain a RanBP2 homologue and that RanBP2 in mammals is not ubiquitously expressed argue against a general role in nucleocytoplasmic transport. Interestingly, both RanGAP1 and Rna1p have been proposed to shuttle between the nucleus and cytoplasm (Feng et al., 1999; Matunis et al., 1998), which might be an alternative explanation for their association with NPCs. RanGAP1 has also been found at the mitotic spindle (Matunis et al., 1996), which might be relevant in the context of Ran’s role during NE assembly after mitosis (see below).

The only known RanGEF, RCC1 (for regulator of chromosome condensation 1) in higher eukaryotes and Prp20p in yeast, is a strictly nuclear protein that appears to be associated with chromatin (Seki et al., 1996). Recent data suggest that RCC1 is anchored on DNA by histones H2A and H2B (Nemergut et al., 2001). However, Fontoura et al. have...
recently reported a pool of RCC1 at the NPC, suggesting that RCC1 has alternative localizations (Fontoura et al., 2000). RCC1 has a highly repetitive primary structure, exhibiting a seven-bladed β-propeller fold similar to that of the β-subunits of heterotrimeric G proteins (Renault et al., 1998), and the recent crystal structure of the RCC1-Ran complex confirms previous suggestions that Ran binds to one face of the propeller (Renault et al., 2001). RanGEF interacts mainly with the switch II region of Ran, one of two highly flexible regions of the conserved nucleotide-binding fold of Ras-like GTPases that undergo major conformational changes upon transition between the two nucleotide-bound states (Bourne et al., 1991).

The compartmentalization of RanGAP and RanGEF has two important consequences. First, Ran has to shuttle between the cytoplasm and nucleoplasm in order to complete one round of GTP hydrolysis and guanine-nucleotide exchange. This is evident from the altered steady-state localization of Saccharomyces cerevisiae Ran, Gsp1p, in cells carrying loss-of-function mutations in the genes encoding the yeast homologues of RanGAP1 (rnlal-1) and RCC1 (prp20-1). The predominantly nuclear signal of Gsp1p in wild-type cells is enhanced in the rnal-1 mutant but not in the prp20-1 mutant (Fig. 1; Stochaj et al., 2000). The growth defect of a prp20-1 mutant at restrictive temperature can be partially suppressed by overexpression of Gsp1p, which suggests that the nuclear concentration of Ran is critical for yeast cell viability (Belhumeur et al., 1993; Kadowaki et al., 1993). Second, the two nucleotide-bound states of Ran are asymmetrically distributed with respect to the NE: the predominant form in the cytoplasm is RanGDP, whereas the predominant form in the nucleoplasm is RanGTP. This steep RanGTP gradient across the nuclear envelope is thought to impart directionality on nucleocytoplasmic transport (see below).

Ran targets

NTF2 and related proteins

NTF2 (nuclear transport factor 2; p10) was identified with Ran in a biochemical fraction required for active nuclear import of a model substrate in digitonin-permeabilized cells (Moore and Blobel, 1994). The protein was shown to play an essential role in replenishing the nucleus with Ran by acting as a nuclear import receptor for Ran both in permeabilized cell systems (Ribbeck et al., 1998; Smith et al., 1998; Steggerda et al., 2000) and in vivo (Quimby et al., 2000a; Quimby et al., 2000b). NTF2, which has a molecular weight of only 10 kDa, forms a homodimer and binds directly and specifically to two molecules of Ran in its GDP-bound form. The crystal structure of the protein complex reveals direct contacts between the hydrophobic cavity of NTF2 and the switch II region of RanGDP (Stewart et al., 1998). Independently of its binding to RanGDP, NTF2 interacts with FG-repeat-containing nucleoporins (Clarkson et al., 1996; Paschal and Gerace, 1995) and localizes to NPCs at steady state (M.K. and E.H., unpublished). The interactions between NTF2 and both RanGDP and FG repeats are essential for nuclear import (Bayliss et al., 1999; Clarkson et al., 1997; Paschal et al., 1997; Wong et al., 1997). The overlap of NTF2-binding sites on the NPC with those for the prototypical nuclear transport receptor, importin β, might explain the observed interference between NTF2 and the classical nuclear import route, which is mediated

Fig. 1. (A) The Ran GTPase cycle. GTP hydrolysis and guanine-nucleotide exchange on Ran occur in the cytoplasm and the nucleus, respectively, owing to the localization of the only known Ran-specific GTPase-activating protein (GAP) and guanine-nucleotide-exchange factor (GEF). Additional Ran-binding proteins, RanBP1 and Mog1, coactivate the reactions as indicated. Nuclear import and export of Ran through the nuclear pore complexes (NPCs, shown in grey) are accomplished by the RanGDP-binding NTF2 protein and the family of RanGTP-binding karyopherins (Imp/Exp), respectively. (B) Dynamic steady-state localization of Ran (Gsp1p) visualized in S. cerevisiae. A low-copy number plasmid encoding GFP-Gsp1p under control of the NOP1 promoter (Lau et al., 2000) was introduced into temperature-sensitive S. cerevisiae strains defective for RanGAP (rnal-1) and RanGEF (prp20-1) and an isogenic wild-type strain. Fluorescence microscopy was performed as described (Lau et al., 2000).
Table 1. Known Ran-binding proteins

<table>
<thead>
<tr>
<th>Protein class (S. cerevisiae homolog)</th>
<th>Binding specificity (experimental evidence)</th>
<th>Steady-state localization</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>RanGAP1 (Rna1p)</td>
<td>RanGTP (iv)</td>
<td>Cytoplasm and NPC</td>
<td>RanGAP</td>
</tr>
<tr>
<td>RCC1 (Prp20p)</td>
<td>RanGDP, RanGTP, empty Ran (iv)</td>
<td>Nucleus and NPC</td>
<td>RanGEF</td>
</tr>
<tr>
<td>NTF2/p10/pp15 (NTF2)</td>
<td>RanGDP (iv)</td>
<td>NPC</td>
<td>Nuclear RanGDP import</td>
</tr>
<tr>
<td>NXT1/p15 (na)</td>
<td>RanGTP (iv)</td>
<td>Nucleus and NPC</td>
<td>Nuclear export</td>
</tr>
<tr>
<td>Nup358/RanBP2 (na); Nup153 (na)</td>
<td>RanGDP (iv)</td>
<td>Cytoplasmic and nuclear face of NPC, respectively</td>
<td>Structural components of the NPC, nuclear import and export</td>
</tr>
<tr>
<td>Mog1 (Mog1p)</td>
<td>RanGDP, RanGTP, empty Ran (iv)</td>
<td>Nucleus</td>
<td>Guanine-nucleotide exchange on Ran</td>
</tr>
<tr>
<td>Dis3 (Dis3p)</td>
<td>RanGDP, RanGTP, empty Ran (iv)</td>
<td>Nucleolus</td>
<td>Intranuclear RNA processing and degradation</td>
</tr>
<tr>
<td>Karyopherins</td>
<td>RanGTP (iv)</td>
<td>Nucleus, cytoplasm, NPC</td>
<td>Nuclear export of RanGTP and cargo, nuclear import of cargo</td>
</tr>
<tr>
<td>RanBP1 (Yrb1p), Nup358/RanBP2 (na), RanBP3 and Nup50 (Yrb2p, Nup2p)</td>
<td>RanGTP (iv)</td>
<td>Cytoplasmic, NPC and nucleus, respectively</td>
<td>GTP hydrolysis on Ran, guanine-nucleotide exchange on Ran, nuclear import and export</td>
</tr>
<tr>
<td>RanBPM (Yrb30p)</td>
<td>RanGTP (2H)</td>
<td>Centrosome</td>
<td>Mitosis</td>
</tr>
<tr>
<td></td>
<td>RanGTP (iv)</td>
<td>Cytoplasm</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Abbreviations: iv, in vitro binding; 2H, two-hybrid interaction; NPC, nuclear pore complex; na, not applicable.

by an importin αβ heterodimer, in vitro (Lane et al., 2000; Paschal et al., 1996; Tachibana et al., 1996).

The recent crystal structure of the RCC1-Ran complex (Renault et al., 2001) suggests that NTF2 and RCC1 cannot bind simultaneously to RanGDP and, thus, RanGDP has to dissociate from NTF2 for nucleotide exchange. Such a conclusion is consistent with the reported guanine-nucleotide-dissociation inhibitor (GDI) activity of NTF2 (Yamada et al., 1998) and the need for nuclear RanGTP-binding proteins, in addition to NTF2, if efficient nuclear accumulation of Ran is to occur in a permeabilized cell system (Ribbeck et al., 1998). These findings suggest that release and guanine-nucleotide exchange on RanGDP in the nucleus require additional factors. These factors would also drive the guanine-nucleotide-exchange reaction in the right direction, since RCC1 has no intrinsic preference for either of the two nucleotide-bound states of Ran. Possible candidates for such factors are RanBP1 and Mog1p (see below).

Recently, Katahira et al. identified a protein that has significant similarity (26% sequence identity) to NTF2, NXT1 (NTF2-related nuclear export protein 1; p15) in communoprecipitation experiments with TAP, an essential mRNA export factor in mammalian cells (Katahira et al., 1999). Interestingly, NXT1 forms a heterodimer with a domain of TAP that shows similarity to NTF2 (Strässer et al., 2000; Suyama et al., 2000). It can coactivate TAP-dependent RNA export both in yeast and in mammalian cells (Braun et al., 2001; Guzik et al., 2001; Katahira et al., 1999) but also enhances a variety of other nuclear export pathways, which suggests that it is a general nuclear export factor (Black et al., 1999; Ossareh-Nazari et al., 2000). In accordance with such a role, NXT1/p15 has been localized to the nucleoplasm and the NPC at steady state and shown to shuttle between the nucleus and the cytoplasm (Black et al., 1999). Like NTF2, NXT1 binds directly to Ran, however, in contrast to NTF2 binding, binding of NXT1 is apparently specific for the GTP-bound form of Ran. This interaction, although controversial because it has been observed only in one laboratory so far, appears to be important for the role of NXT1 in RNA export (Ossareh-Nazari et al., 2000). The exact function of NXT1 in nuclear export is unclear. In Crm1-dependent nuclear protein export, NXT1 facilitates the terminal release of the export complexes from the NPC in the cytoplasm (Black et al., 2001), which suggests it is involved in late steps of this process.

RanGDP-binding Zn-finger nucleoporins

Besides NTF2, only two other RanGDP-binding proteins have been identified, Nup358/RanBP2 and Nup153 (Nakiely et al., 1999; Saitoh et al., 1996; Yaseen and Blobel, 1999b). Both proteins are components of the NPC and are thus probably responsible for the association of RanGDP with the NPC (Görlich et al., 1996). Nup358/RanBP2 and Nup153 bind to RanGDP through a common Zn-finger motif that has also been implicated in the interaction with Crm1, a nuclear export receptor of the importin β family, and DNA, respectively (Singh et al., 1999; Sukenaka and Blobel, 1993). Interestingly, the two nucleoporins are located on different sides of the NPC, Nup358/RanBP2 being a component of the cytoplasmic filaments and Nup153 localizing to the nuclear basket of the NPC. The exact role of these RanGDP-binding sites on both sides of the NPC is not clear. The implication of both nucleoporins in nuclear import and export suggests that the RanGDP-binding domains are involved in the fine tuning of the Ran GTPase cycle for nucleocytoplasmic transport, although this has not yet been addressed experimentally (Kehlenbach et al., 1999; Shah et al., 1998; Singh et al., 1999; Yaseen and Blobel, 1999a). Alternatively, these domains could play a role in Ran-dependent NE formation during mitosis in higher eukaryotes (see below). In accordance with such a hypothesis, both Nup358/RanBP2 and Nup153 have been shown to be among the first nucleoporins incorporated in the newly forming NE after chromosome segregation (Bodoor et al., 1999; Haraguchi et al., 2000). Indeed, yeasts, which do not undergo nuclear breakdown during mitosis, do not have Zn-finger-domain-containing nucleoporins.

The RanBP1 family of RanGTP-binding proteins

RanBP1 (Ran-binding protein 1) was the first Ran-binding protein identified (Coutavas et al., 1993; Table 1). The protein binds specifically to RanGTP through a highly conserved ~140-residue Ran-binding domain (RBD; also known as RanBP1-homologous domain, RBH; Beddow et al., 1995;
Lounsbury et al., 1994). This domain is also found in the giant nucleoporin Nup358/RanBP2 (see above), in which several copies are present, and in a group of nuclear proteins represented by Nup2p and Yrb2p in <i>S. cerevisiae</i>. Homologues of the latter group of proteins include Hba1p in <i>Shizosaccharomyces pombe</i> and Nup50 and RanBP3 in humans (Guan et al., 2000; Mueller et al., 1998; Smitherman et al., 2000; Turi et al., 1996; Welch et al., 1999).

Human RanBP1 and its <i>S. cerevisiae</i> ortholog, Yrb1p, shuttle rapidly between the nucleus and the cytoplasm and localize exclusively to the cytoplasm at steady state (Künzler et al., 2000; Pfal kter and Macara, 2000). Interestingly, nuclear export of RanBP1 depends on Crm1 in both organisms, although the export receptor might recognize different export signals. The nuclear function of RanBP1 remains largely obscure (see below), but the protein is thought to play an important role in the dissociation of export complexes and the recycling of import receptors in the cytoplasm. RanBP1 and isolated RBDs were shown to inhibit exchange but to coactivate hydrolysis of Ran-bound GTP in vitro (Bischoff et al., 1995). More importantly, these domains can overcome the inhibition of GTP hydrolysis imposed by formation of complexes between RanGTP and members of the importin β family of nuclear transport receptor (Bischoff and Görlich, 1997; Floer et al., 1997; Lounsbury and Macara, 1997; see below). The dissociation of such complexes is a crucial step both in the release of cargo from nuclear export complexes and in the recycling of nuclear import receptors since export and import receptors of the importin β family leave the nucleus in complex with RanGTP (Fig. 1A; see below). Interestingly, the level of RanBP1 in mammalian cells is cell cycle regulated, increasing from S phase to M phase, peaking in metaphase and abruptly declining in late telophase (Guarguagliani et al., 2000).

This cycling might reflect the role of Ran during mitosis in higher eukaryotes (see below), given that there is no evidence for such regulation in yeast, in which the NE does not disassemble during mitosis (Bäumer et al., 2000).

Nup358/RanBP2 contains several functional domains, including RanGDP-binding Zn-finger domains (see above) and several RanGTP-binding RBDs. The latter, maybe in conjunction with the RanGDP-binding Zn-finger domains, presumably have the same role as the cytoplasmic RanBP1 (Kehlenbach et al., 1999). The additional function of Nup358/RanBP2 as docking site for RanGAP1 (see above) may further facilitate this function. In the case of the classical nuclear import pathway, the RBDs of Nup358/RanBP2 have been shown to link initiation and termination steps (Yaseen and Blobel, 1999a). As in the case of Zn-finger domains, the RBDs might play an additional role in nuclear envelope formation during mitosis. The structure of a co-crystal containing a RBD of RanBP2 and RanGppNHp revealed a unique molecular embracement between the N-terminal domain of the RBD and the C-terminal domain of Ran (Vetter et al., 1999a). The core domain of the RBD that makes direct contacts with the switch I region of Ran exhibits the same topology as pleckstrin homology (PH) domains implicated in the binding of phosphoinositide lipids, which indicates that this fold represents a versatile interaction module.

The RBDs of nuclear RanBP1-family proteins, exemplified by <i>S. cerevisiae</i> Nup2p and Yrb2p, are rather degenerate, and their affinities for RanGTP are low – in fact, Mueller et al. proposed that is a prerequisite for their function in the nucleus (Mueller et al., 1998). However, there is recent evidence that these proteins, although nuclear at steady state, shuttle between the nucleus and cytoplasm (Dilworth et al., 2001; Lindsay et al., 2001). Both Nup2p and Yrb2p contain FG repeats, as do some of the nucleoporins. These repeats have an affinity for various nuclear transport receptors. Nup2p and Yrb2p have been implicated in Cse1p- and Crm1p-dependent export, respectively, although their exact role in these processes is unclear (Booth et al., 1999; Hood et al., 2000; Solsbacher et al., 2000; Taura et al., 1998). Identifying a possible mechanism, Lindsay et al. have recently shown that RanBP3, the mammalian homologue of Yrb2p, enhances Crm1-mediated nuclear export by increasing the affinity of the receptor for its cargo (Lindsay et al., 2001).

**Karyopherins**

The family of importin-β-like nuclear transport receptors (karyopherins) comprises 14 different members in <i>S. cerevisiae</i> (Adam, 1999). The number of mammalian proteins known to belong to this family is steadily increasing. Members of this family have a repetitive structure that accommodates binding to RanGTP, cargo and the NPC (Mattaj and Conti, 1999). They contain a degenerate ~150-residue RanGTP-binding motif at the N-terminus, which shows no similarity to the RBD of RanBP1. Crystal structures of two importins reveal an arch-like overall structure in which the inner face of the arch binds to RanGTP or cargo in a mutually exclusive manner, and the outer face of the arch makes contacts with FG-repeat sequences in nucleoporins (Bayliss et al., 2000; Chook et al., 1999).

Binding sites for importin β and the RBD on RanGTP do not overlap, which is consistent with the formation of trimeric complexes involving these proteins in vitro (Chi et al., 1997; Vetter et al., 1999b). Such complexes are possible intermediates in the dissociation of karyopherin-RanGTP complexes (see above). Binding of RanGTP to karyopherins thereby not only inhibits RCC1-mediated guanine-nucleotide exchange and RanGAP1-mediated GTP hydrolysis on Ran but also modulates the interaction of karyopherins with cargo and the NPC. In the case of importins, binding of RanGTP releases cargo and the NPC, whereas in the case of exportins binding is required for their association with cargo and the NPC. It will be interesting to see how such similar proteins can exhibit such a different behaviour.

**Mog1 and Dis3**

Oki and Nishimoto identified Mog1p (multicopy suppressor of gsp1) in a genetic screen for multicopy suppressors of temperature-sensitive mutations in <i>GSP1</i>, which encodes yeast Ran (Oki and Nishimoto, 1998). Mog1p is a nuclear protein that is evolutionarily conserved (Marfatia et al., 2001; Tatebayashi et al., 2001). It binds to both nucleotide-bound forms of Ran and competes with NTF2 for binding to RanGDP (Stewart and Baker, 2000). The crystal structure reveals a unique fold, although the topology of some elements resembles part of NTF2 (Stewart and Baker, 2000). <i>mog1</i>-null <i>S. cerevisiae</i> mutants exhibit a temperature-sensitive phenotype that is suppressed by high-copy numbers of <i>NTF2</i> and <i>GSP1</i> (Oki and Nishimoto, 1998). The mutant exhibits defects in nuclear protein import but not in the nuclear export of poly(A)^+RNA (Oki and Nishimoto, 1998). In <i>S. pombe</i>,...
Mog1p is essential for viability, and a temperature-sensitive mutation leads to defects in nuclear protein import and nuclear accumulation of poly(A)^+RNA (Tatebayashi et al., 2001). Overexpression of S. pombe Ran, Sp1p, rescues S. pombe mog1Δ cells from death. Remarkably, the S. pombe knockout is complemented by Xenopus laevis and S. cerevisiae Mog1p, which suggests that the function of Mog1p is conserved from yeast to frog (Nicolas et al., 2001).

At present, the role of Mog1 in the Ran GTPase cycle is unclear. The genetic interaction with NTF2 suggests that Mog1 plays a role in the nuclear accumulation of Ran. Indeed, Gsp1p is already relocalized to the cytoplasm in a mog1Δ strain at the permissive temperature (Stochaj et al., 2000), which is similar to the situation in prp20-1 cells (Fig. 1B). Thus, an increase in the cellular level of Ran is able to rescue three different mutations in S. cerevisiae (prp20-1, ntf2 and mog1Δ) that are all defective in nuclear accumulation of Gsp1p. These findings, together with the fact that Mog1 is located in the nucleus and binds to Ran irrespectively of the nucleotide-bound state (and also binds to the nucleotide-free state), suggest that the protein plays a role in the release of RanGDP from NTF2 and in the subsequent GDP-to-GTP exchange on Ran by RCC1. In accordance with such a hypothesis, two groups recently demonstrated that Mog1 acts as a guanine-nucleotide-release factor for Ran in vitro (Oki and Nishimoto, 2000; Steggerda and Paschal, 2000). However, this activity was on GTP-to-GDP exchange and not the physiological inverse reaction. In fact, Mog1 alone inhibits the latter reaction. Interestingly, most recent results suggest that RanBPM can relieve this inhibition and contribute to cooperative coactivation of RCC1-mediated GDP-to-GTP exchange on Ran, which is similar to the role of RanBPM in RanGAP1-mediated GTP hydrolysis (Nicolás et al., 2001). In accordance with such a model, mutations in YRB1 and PRP20 are conditionally lethal with a deletion of MOG1 (M.K. and E.H., unpublished). This model would also explain the nucleocytoplasmic shuttling of RanBPM.

Dis3p has Ran-binding characteristics similar to those of Mog1p (Noguchi et al., 1996; Shiomi et al., 1998). It was identified in a two-hybrid screen using human Ran as a bait and is conserved from yeast to man. In vitro, Dis3p binds to both nucleotide-bound forms of Ran but also to the nucleotide-free form of Ran. In addition, it can enhance the RanGTP activity of RCC1 and is found in complex with Ran and RCC1 in vivo (Noguchi et al., 1996). Recently, Mitchell et al. reported that Dis3p is identical to Rrp44p, a component of a nucleolar RNA-processing complex called the exosome (Mitchell et al., 1997). Dis3p/Rrp44p itself has 3′-5′ exoribonuclease activity, but it is unclear how this activity relates to its in vitro activity as a coactivator of RCC1-mediated guanine-nucleotide exchange on Ran. Suzuki et al. recently proposed that Ran regulates exosome assembly/disassembly through Dis3p independently of its role in nucleocytoplasmic transport (Suzuki et al., 2001). In any case, mutations in the essential S. cerevisiae DIS3 gene lead to nuclear accumulation of various RNAs, which might be a consequence of defects in their processing (Gadal et al., 2001; Grosshans et al., 2001).

RanBPM and Yrb30p

Nakamura et al. isolated RanBPM in a two-hybrid screen using Ran as a bait (Nakamura et al., 1998). They showed that RanBPM binds specifically to the GTP-bound form of Ran in the two-hybrid system. To date, no direct binding of RanBPM to Ran in vitro has been demonstrated. Interestingly, the protein localizes to centrosomes and its overexpression induces ectopic nucleation of microtubules. This phenotype, in conjunction with the two-hybrid interaction with Ran, suggests that RanBPM is a target of Ran in microtubule aster formation (see below). RanBPM displays considerable similarity to the C-terminal half of an S. cerevisiae ORF, but the relevance of this similarity remains to be shown (Nakamura et al., 1998).

Yrb30p is an S. cerevisiae ORF that was identified in a two-hybrid screen using the hydrolysis-defective Gsp1pG21V mutant as a bait (A. Braunwarth, M. Fromont-Racine, P. Legrain, R. Bischoff, E.H. and M.K., unpublished). The protein interacts specifically with the GTP-bound form of Gsp1p in vitro. Interestingly, Yrb30p does not show any sequence similarity to either RanBP1 or importin β and appears to be localized exclusively to the cytoplasm. The cellular function of this novel RanGTP-binding protein is unknown.

The multiple functions of Ran

Nucleocytoplasmic transport

Since the identification of Ran as a protein necessary for nuclear protein import (Moore and Blobel, 1993), the field of nucleocytoplasmic transport has boomed. As a result, today we have a relatively precise understanding of the mechanism of this essential cellular process (Görlich and Kutay, 1999; Nakielný and Dreyfuss, 1999; see animation in; Allen et al., 2000). Ran plays an eminent role in this process, helping to establish compartmental identity and providing the directionality of transport (Macara et al., 2000). In contrast to early hypotheses, the current view is that GTP hydrolysis on Ran is not required as an energy source for the translocation process. There is an asymmetric distribution of RanGTP (and RanGDP) across the nuclear envelope (see above), and directionality of transport is provided by the different modulation of importins and exportins by RanGTP with respect to their cargo binding. Whereas importins bind to RanGTP and cargo in a mutually exclusive manner, exportins bind to RanGTP and cargo cooperatively. As a consequence, formation of importin-cargo and exportin-cargo-RanGTP complexes is restricted to the cytoplasm and the nucleoplasm, respectively. Indeed, inversion of the RanGTP gradient in vitro leads to an inversion of the directionality of nucleocytoplasmic transport (Nachury and Weis, 1999). At this point, we would like to emphasize that, among all Ran-binding proteins discussed above, the family of importin-β-like nuclear import and export receptors represents the only direct link between the Ran GTPase cycle and a cellular process. In this strict sense, members of this family of proteins represent the only real Ran targets known so far.

Spindle formation

The identification of a centrosomal protein, RanBPM, as a Ran-binding protein that induces ectopic microtubule nucleation when overexpressed suggested a possible role for Ran in chromosome segregation (see above). Studies using mitotic extracts from X. laevis eggs have shown that Ran is necessary for centriole-dependent microtubule aster formation, can stabilize microtubule asters and even promote spindle formation when it is exogenously added (Kahana and...
Cleveland, 1999). Interestingly, this activity of Ran, which occurs in the absence of nuclei and is thus independent of its role in nucleocytoplasmic transport, relies on the GTP-bound form of the GTPase. GTP hydrolysis does not seem to be important, because mutant forms of RanGTP that cannot hydrolyze GTP can do the job. These activities, however, require other proteins in the egg extract: RanGTP cannot stimulate microtubule polymerization from purified tubulin subunits. Such proteins include motor proteins (dynein) and other centrosome-associated factors (NuMA, TPX2, XGRIP109 and XMAP215).

The mechanism of this additional function of Ran had remained obscure until recently, but three groups have now reported that, in these extracts, RanGTP acts by releasing microtubule-associated proteins (MAPs) TPX2 and NuMA from importin β (Gruss et al., 2001; Nachury et al., 2001; Wiese et al., 2001). This suggests that the role of RanGTP in microtubule nucleation is very similar to its role in nucleocytoplasmic transport. In both cases, high local concentrations of RanGTP, either close to chromatin (due to chromatin-bound RCC1) or within the nucleus, release import cargo from their receptor. During mitosis, the MAPs released in such a way are able to trigger microtubule nucleation owing to the absence of an NE. During interphase, these proteins are sequestered away from the cytoplasmic tubulin into the nucleus possibly in order to prevent premature microtubule nucleation. It has been speculated that RanGTP-induced spindle formation is important in plants and in some meiotic cells that lack a defined microtubule-organizing center like a centriole. However, there is evidence that Ran and importin β perform mitotic functions even in somatic cells (Guarguaglini et al., 2000; Nachury et al., 2001), which argues for a more general mechanism.

Nuclear envelope formation

Besides its role in nucleocytoplasmic transport and spindle formation, Ran is also required for NE assembly in chromatin-free Xenopus egg extracts (Hetzer et al., 2000; Zhang and Clarke, 2000) and human somatic cell extracts (Zhang and Clarke, 2001). In contrast to microtubule nucleation, NE assembly requires both GTP hydrolysis and guanine-nucleotide exchange on Ran, since non-hydrolyzable GTP analogues and mutants of Ran incapable of GTP hydrolysis (RanQ69L) or nucleotide-binding (RanT24N) inhibit this process. In addition, depletion of RCC1 and RanGAP1 from the extracts abolishes NE assembly. The Ran-induced NE is continuous and contains NPCs that support active nuclear import and export. The molecular mechanism involved is currently not known, because no targets of Ran in this process have been identified to date. The RanGDP- and/or RanGTP-binding nucleoporins are candidates for such proteins (see above). A high local concentration of RanGTP on chromatin appears to promote recruitment of membrane vesicles, and a localized Ran GTPase cycle accomplished by RCC1 and RanGAP1 on chromatin then leads to vesicle fusion.

Conclusions

It is clear now that Ran functions in at least three different processes: nucleocytoplasmic transport, microtubule aster formation and NE formation. Although the molecular details of its roles in latter two processes are still obscure, all appear to depend on the localized generation of RanGTP by chromatin-bound RCC1. Remarkably, the latest reports now indicate that all these functions could rely on the same class of target protein, karyopherins. Intriguingly, these proteins represent, in a strict sense, the only direct Ran-targets known so far. Weis and co-workers have speculated that the function of these proteins in microtubule aster and NE formation preceded their function in nucleocytoplasmic transport (Nachury et al., 2001). Alternatively, the additional functions of Ran during open mitosis in higher eukaryotes might have evolved from the situation in fungi, in which proteins involved in spindle formation have to be imported into the nucleus because the NE does not disassemble during mitosis (Wiese et al., 2001).

It will be interesting to see whether importin β also plays a role in NE formation and whether there are Ran targets in microtubule aster formation and NE formation, besides importin β, that are specific for these cellular processes. Finally, it would be interesting to know whether these additional roles of Ran are restricted to eukaryotic organisms that exhibit open mitosis or whether there are similar roles in fungi that do not undergo nuclear breakdown during mitosis. In metazoans at least, Ran can be regarded as a marker for the nuclear compartment during interphase, as a marker for condensed chromatin during metaphase and anaphase and as a marker for decondensed chromatin during telophase (Hetzer et al., 2000).

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