The Mammalian Mog1 Protein Is a Guanine Nucleotide Release Factor for Ran*

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Ran is a Ras-related GTPase that is essential for the transport of protein and RNA between the nucleus and the cytoplasm. Proteins that regulate the GTPase cycle and subcellular distribution of Ran include the cytoplasmic GTPase-activating protein (RanGAP) and its co-factors (RanBP1, RanBP2), the nuclear guanine nucleotide exchange factor (RanGEF), and the Ran import receptor (NTF2). The recent identification of the Saccharomyces cerevisiae protein Mog1p as a suppressor of temperature-sensitive Ran mutations suggests that additional regulatory proteins remain to be characterized. Here, we describe the identification and biochemical characterization of murine Mog1, which, like its yeast orthologue, is a nuclear protein that binds specifically to RanGTP. We show that Mog1 stimulates the release of GTP from Ran, indicating that Mog1 functions as a guanine nucleotide release factor in vitro. Following GTP release, Mog1 remains bound to nucleotide-free Ran in a conformation that prevents rebinding of the guanine nucleotide. These properties distinguish Mog1 from the well characterized RanGEF and suggest an unanticipated mechanism for modulating nuclear levels of RanGTP.

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The abbreviations used are: NPC, nuclear pore complex; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GST, glutathione S-transferase; ORF, open reading frame; NES, nuclear export signal; NLS, nuclear localization signal; TB, transport buffer; ts, temperature-sensitive; RanGTP, Ran guanine nucleotide exchange factor; RanGAP, Ran GTPase-activating protein; RanBP1, Ran-binding protein 1; GST, glutathione S-transferase; PAG, polyacrylamide gel electrophoresis; FPLC, fast protein liquid chromatography. For the importin/karyopherin superfamily of transport receptors, which escort NLS or NES cargo proteins through the NPC. Import receptors bind NLS cargo in the cytoplasm, and the import complex translocates to the nucleus, where RanGTP stimulates release of NLS cargo. In contrast, export receptors bind NES cargo in the nucleus together with RanGTP. Translocation of the receptor-NES cargo-RanGTP export complex to the cytoplasm is succeeded by GTP hydrolysis, which triggers disassembly of the complex and release of NES cargo.

The nucleotide state and subcellular distribution of Ran, both of which are critical for its ability to coordinate transport, are controlled by regulatory proteins (6–9). The generation of RanGTP occurs only in the nucleus by a chromatin-bound guanine nucleotide exchange factor (RanGEF) that catalyzes GDP release and GTP binding (10–12). Conversion of RanGTP to RanGDP occurs in the cytoplasm and is mediated by an NPC-associated GTPase-activating protein (RanGAP) and its co-activators, RanBP1 and RanBP2 (13–20). The appropriate nucleocytoplasmic distribution of Ran is maintained by a balance between nuclear export of RanGTP, in association with transport receptors, and nuclear import of RanGDP mediated by NTF2 (9, 21–22). The high concentration of Ran in the nucleus and the mutually exclusive subcellular distribution of RanGAP and RanGEF have led to the hypothesis that a gradient of RanGTP exists across the nuclear envelope (7, 8). This gradient is thought to confer compartment identity to the nucleus and the cytoplasm by specifying the Ran-dependent assembly and disassembly of transport complexes in the appropriate subcellular location. Therefore, the high concentration of RanGTP in the nucleus should favor assembly of export complexes and disassembly of import complexes (1, 2). This hypothesis is analogous to, for example, the compartment identity of the endosome, where a proton-rich environment favors disassembly of receptor-ligand complexes.

Although the proteins that modulate the nucleotide state and subcellular distribution of Ran (RanGAP, RanGEF, and NTF2) have provided a basis for understanding Ran function in nuclear transport, the recent identification of the Saccharomyces cerevisiae protein Mog1p suggests that the regulation of Ran is more complex. Mog1p was identified as a suppressor of certain temperature-sensitive (ts) alleles of Ran and was shown to bind specifically to RanGTP (23). Analysis of a ∆mog1 strain demonstrated that Mog1 is required for nuclear protein import in vivo. Mog1 displays a genetic interaction with the Ran import receptor, NTF2, based on the suppression of ts growth of a ∆mog1 strain by overexpression of NTF2 (23). Although the function of Mog1p in nuclear transport is unknown, the multiple genetic and biochemical interactions between Mog1, NTF2, and Ran suggest that Mog1 may regulate the subcellular distribution or GTPase cycle of Ran.

Here, we report the identification and biochemical charac-
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**Fig. 1.** Primary structure and subcellular localization of Mog1. A, alignment of Mog1 proteins from *M. musculus*, *S. cerevisiae*, and *S. pombe* (GenBank™ accession numbers AF243512, BAA28825, and CAA20124, respectively). Positions of amino acid identity are shaded. Mouse Mog1 is 24 and 29% identical to its budding and fission yeast orthologues, respectively. B, immunofluorescence microscopy showing the nuclear localization of Mog11 in BHK21 cells. A plasmid encoding Mog1 with an N-terminal epitope tag (Flag-Mog1) was introduced into cells using the calcium phosphate method, and the protein localization was examined 48 h post-transfection (left panel). The cells were counter-stained with 4',6-diamidino-2-phenylindole (DAPI) to reveal the position of nuclei within the field (right panel).

EXPERIMENTAL PROCEDURES

Recombinant Methods—The plasmid encoding murine Mog1 was obtained from the IMAGE consortium (clone I.D. no. 736209) and sequenced using T7 and T3 primers to define the complete open reading frame (ORF). The DNA and protein sequences of murine Mog1 were deposited in GenBank™ (accession no. AF243512). The ORF was amplified by polymerase chain reaction and cloned into bacterial and eukaryotic expression vectors. Recombinant Mog1 was expressed as a second guanine nucleotide release factor for Ran in the cell. Release of GTP from Ran and inhibition of the release activity by the RanGTP-binding protein RanBP1 are biochemical properties shared by Mog1 and RanGEF. Unlike RanGEF, however, Mog1 remains bound to nucleotide-free Ran in a complex that prevents nucleotide binding to Ran. Mog1 may provide a mechanism for regulating the concentration of RanGTP in the nucleus.

RESULTS

Identification of the Murine Orthologue of Mog1—We used the protein sequence of *S. cerevisiae* Mog1p (GenBank™ accession no. BAA28825) in BLAST searches of GenBank to identify potential homologues in other species. We identified ORFs...
in mouse and Schizosaccharomyces pombe with expectation values (10^{-7}-10^{-10}) indicating that these proteins are evolutionarily related to S. cerevisiae Mog1p. We determined the complete ORF of murine Mog1 by sequencing EST clone AA270237 and aligned it with the fungal orthologues (Fig. 1A).

The MOG1 genes from S. cerevisiae, S. pombe, and Mus musculus encode proteins of 24, 22, and 20 kDa, respectively, and share 24–29% amino acid identity. Murine Mog1 is 24% identical to S. cerevisiae Mog1p and 29% identical to S. pombe Mog1p, whereas S. pombe Mog1p is 28% identical to S. cerevisiae Mog1p. An alignment of the three Mog1 proteins reveals that the S. cerevisiae protein contains an N-terminal extension of 29 residues that is not found in the mouse or fission yeast protein (Fig. 1A). Yeast contain a single MOG1 gene, and Mog1 proteins do not display significant sequence similarity with proteins other than Mog1 orthologues.

**Mog1 Is a Nuclear Protein**—To determine the cellular distribution of Mog1, we expressed epitope-tagged murine Mog1 protein in BHK21 cells and examined its localization by immunofluorescence microscopy. We found that Mog1 is highly concentrated in the nucleus but is excluded from nucleoli and shows no localization at the nuclear envelope (Fig. 1B). S. cerevisiae Mog1 has been shown to be a nuclear protein as well (23).

**Mog1 Is a Ran-binding Protein**—As a first step in our biochemical characterization of murine Mog1, we expressed and purified a recombinant version of the protein and tested it for Ran binding using GST pull-down assays. S. cerevisiae Mog1 has been shown to bind to RanGTP in vitro (23). GST-Mog1 and GST were immobilized on glutathione beads and incubated with whole cell lysate from BHK21 cells. The bound fractions were examined by immunoblotting using anti-Ran antibody. GST-Mog1, but not GST, precipitated Ran from cell lysate, implying that Mog1 is a Ran-binding protein. To determine whether the Mog1-Ran interaction reflects direct binding, we performed the GST pull-down assay using recombinant Ran in place of cell lysate. Ran was preloaded with radiolabeled GTP to quantitate the interaction, and a GST fusion of the RanGTP-binding protein RanBP1 was included as a positive control. RanGTP binding to GST-Mog1 was similar to the level of binding to GST alone (Fig. 2B), whereas 3.7-fold more RanGTP bound to GST-RanBP1 than GST. One interpretation of these results is that Mog1 binding to Ran requires the presence of a factor present in cell lysate. We found, however, that recombinant Ran could bind stably to Mog1 as well as to another Ran-binding protein, RCC1, included as a positive control (Fig. 2C). As demonstrated below, the apparent discrepancy in Ran binding in these two experiments can be explained by the readout. That is, the Ran binding assay that relies on measuring radiolabeled GTP fails to register a positive interaction because the nucleotide bound to Ran is released into solution by Mog1.

The ability of Mog1 to bind to RanGTP was further examined using a mutant form of Ran that binds stably to GTP (Ran Q69L (15)). Mog1 displayed a very low level of binding to Ran Q69L as compared with the control RanGTP-binding protein, RanBP1 (Fig. 2D). GST and NTF2, the latter of which interacts with the GDP form of Ran, did not bind detectable levels of Ran Q69L. This result indicates that Mog1 and RanBP1 bind to distinct domains of Ran. The fact that Mog1 binding is reduced by the Q69L mutation implicates the switch II region of Ran in the Mog1-Ran interaction.

**Mog1 Is a Guanine Nucleotide Release Factor for RanGTP**—Our observation that Ran is recovered in a complex with Mog1 under conditions in which the [α-32P]GTP label is not recovered suggested a protein-protein interaction that alters the guanine nucleotide-bound state of Ran. The possibility that Mog1 functions as a RanGAP was ruled out by thin layer chromatography, which showed no specific conversion of GTP to GDP).2 The possibility that Mog1 stimulates GTP release from Ran was examined in a filter binding assay. We found that Mog1 assayed at 10 μg/ml (0.5 μM) could stimulate the release of ~80% of the radiolabeled GTP from Ran in a 10-min reaction (Fig. 3A, lower curve). Nucleotide release measured in this assay is specific for GTP, since radiolabel is on the terminal phosphate. The only known protein that can catalyze nucleotide release from RanGTP is RCC1. Because nucleotide release by RCC1 is inhibited by the RanGTP-binding protein RanBP1 (16), we used recombinant RanBP1 to assess whether nucleotide release by RCC1 and Mog1 may be mechanistically similar. Indeed, we found that RanBP1 can protect RanGTP from nucleotide release mediated by Mog1 (Fig. 3A, upper curve). Moreover, we determined that a RanBP1 mutant (E37K) that binds Ran with reduced affinity (26) also has a reduced capacity to protect Ran from Mog1-stimulated nucleotide release (Fig. 3A, middle curve).

**Mog1 Forms a Complex with Ran and RanBP1**—The basis of RanBP1-mediated protection of RanGTP from Mog1-stimulated nucleotide release could be explained by competition be-

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2 S. M. Steggerda and B. M. Paschal, unpublished observations.
proteins were used at a concentration of 0.5 μM of [γ-32P]GTP from Ran in the presence of buffer ( ), wild type RanBP1 ( ), or E37K mutant of RanBP1 ( ). Under these reaction conditions, 250 nM (5 μg/ml) Mog1 protein was sufficient to stimulate release of ~50% of the radiolabeled GTP from Ran (total concentration 125 nM). The RanBP1 proteins were used at a concentration of 0.5 μM. The reactions were incubated for 10 min at 30 °C, and the radioactivity that remained bound to Ran was determined by filter binding and scintillation counting. Error bars represent S.E. of three separate experiments. B and C, RanBP1 protects RanGTP from Mog1 release activity by forming a complex with these proteins. GST, NTF2, NXT1, or GST-Mog1 were adsorbed to microtiter wells and incubated with [γ-32P]GTP-Ran in the absence (B) or presence (C) of GST-RanBP1. Bound proteins were solubilized, and Ran binding was measured by scintillation counting of the release fraction. Values shown are representative of at least three separate experiments; error bars represent S.E. of triplicate wells. D and E, Mog1 does not display nucleotide exchange activity. Nucleotide exchange on RanGTP (125 nM) was assayed as the incorporation of radiolabeled GDP (100 μM [3H]GDP) in the presence of 10 mM EDTA ( ), 30 nM (2 μg/ml) GST-RCC1, or the indicated concentrations of Mog1 ( ) at 30 °C, and measured by filter binding and scintillation counting.

**Mog1 Inhibits Nucleotide-free Ran from Binding Nucleotide**—All known exchange factors for small GTPases stimulate both nucleotide release and nucleotide binding in vitro (27). The biochemical similarities of Mog1 and RCC1 involving nucleotide release and protection by RanBP1 prompted us to examine whether Mog1 also can mediate nucleotide exchange on Ran. We loaded Ran with unlabeled GTP, and measured the incorporation (exchange) of radiolabeled GDP as a function of time in a filter binding assay. Nucleotide exchange promoted by EDTA in this assay is linear during the 30 min reaction (Fig. 3D, upper curve). In contrast, nucleotide exchange was not observed in the presence of Mog1 (Fig. 3D, lower curve). The addition of an excess of Mog1 (40 μg/ml; 2 μM) failed to stimulate nucleotide exchange under conditions in which RCC1 (2 μg/ml; 0.03 μM) catalyzed the GTP-GDP exchange on Ran (Fig. 3E). Taken together, our results indicate that GTP release is stimulated by Mog1, but subsequent binding of GDP is inhibited by Mog1. Thus, under the assay conditions described in this study, Mog1 is a guanine nucleotide release factor but not a guanine nucleotide exchange factor.

**Chromatography of Mog1 Complexes**—We used gel filtration chromatography to analyze the size and composition of complexes formed by recombinant Mog1, RanGTP, and RanBP1 in solution. The proteins (5 μM each) were incubated for 60 min on ice to allow binding and fractionated by FPLC using a Superdex 75 gel filtration column. The elution positions were determined by immunoblotting and by comparison with protein standards. Mog1 chromatographed alone eluted in a single peak in fraction 24, indicating that the ~24-kDa protein is a monomer in solution (Fig. 4A). Ran, which is an ~24-kDa monomer in solution, eluted at a position similar to Mog1 (fractions 23 and 24). Incubation of Mog1 and RanGTP prior to chromatographic separation resulted in the formation of a complex of the two proteins that eluted in fraction 20 (Fig. 4C). This elution position corresponds to ~50 kDa, indicating that the stoichiometry of the Mog1-Ran complex is 1:1.

We also examined whether Mog1, Ran, and RanBP1 can form a complex in solution. We found that RanBP1 assembles into a complex with Mog1 and Ran that eluted in fractions 18 and 19 (Fig. 4D). These fractions correspond to a molecular mass of 59–67 kDa, indicating that the stoichiometry of the Mog1-RanGTP-RanBP1 complex is 1:1:1. To examine whether the complex containing Mog1, RanGTP, and RanBP1 may involve a direct interaction between Mog1 and RanBP1, the elution of the latter proteins was analyzed in the absence of Ran. RanBP1 (23 kDa) when chromatographed alone elutes with a peak in fractions 20–21, indicating that it behaves as a dimer. This observation has been reported by other laboratories (16). The size of the Mog1-RanGTP-RanBP1 complex indicates, how-
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FIG. 4. Binary and ternary complexes of Mog1 resolved by gel filtration chromatography. A–G, recombinant proteins (5 µM each in a total volume of 200 µl) were incubated on ice for 1 h prior to chromatography on a Superdex 75 FPLC column. The eluted proteins were analyzed by immunoblotting. A and B, Mog1 and Ran, when chromatographed separately, elute as monomeric proteins. C, Mog1 and Ran, when chromatographed together, elute as a binary complex with an apparent molecular mass of ~50 kDa (fraction 20). D, Mog1, Ran, and RanBP1, when chromatographed together, elute as a ternary complex with an apparent molecular mass of ~67 kDa (fraction 18). E–G, Mog1 does not display direct interactions with RanBP1 or NTF2, and it does not form a ternary complex with Ran and NTF2.

ever, that only a single RanBP1 is incorporated into the complex. Thus, although RanBP1 behaves as a dimer when chromatographed alone, we find that RanBP1 interacts with Ran and Mog1 as a monomer. We also found that Mog1 and RanBP1 do not directly interact (Fig. 4E), indicating that RanGTP bridges the complex of these proteins both in solution (Fig. 4D) and in the solid phase binding assay (Fig. 3C).

We extended our chromatographic analysis to include NTF2, which displays a genetic interaction with Mog1. High copy expression of NTF2 can suppress ts growth in a Δmog1 strain (23), suggesting a possible physical interaction between NTF2 and Mog1 in vivo. Mog1, Ran, and NTF2 were combined and analyzed by chromatography. We observed the formation of a Mog1-Ran complex (fractions 19 and 20) and an NTF2-Ran complex (fraction 22) but not the formation of a trimeric complex (Fig. 4F). The formation of complexes containing RanGTP and Mog1 and RanGDP and NTF2 reflects the presence of both nucleotide forms of Ran in the sample. In summary, our analysis using recombinant proteins reveals the formation of two distinct Mog1 complexes: Mog1 that is stably bound to nucleotide-free Ran and Mog1 that is stably bound to RanGTP and RanBP1.

DISCUSSION

Because Ran plays an essential role in nucleocytoplasmic transport, defining the function and location of its regulatory proteins is fundamental to understanding the mechanisms of nuclear import and export. The key regulatory proteins for Ran include RanGAP (also called Rna1p (13–15, 17)), RanGAP co-activators (RanBP1 and RanBP2 (16, 18–20)), the Ran import receptor (NTF2 (9, 21, 22, 28, 29)), and RanGEF (also called RCC1 (10–12)). The cytoplasmic localization of RanGAP, RanBP1, and RanBP2 ensures that Ran in the cytoplasm is predominantly GDP-bound, whereas the nuclear localization of RanGEF ensures that Ran in the nucleus is predominantly GTP-bound. Our identification of a release factor for RanGTP indicates, however, that the GTPase cycle involves an additional regulatory protein, Mog1.

Our results have demonstrated that murine Mog1 is a guanine nucleotide release factor that is highly concentrated in the nucleus. Mog1 binds specifically to RanGTP, an interaction that results in release of GTP and formation of a stable complex with the nucleotide-free form of Ran. Surprisingly, the Mog1-Ran complex fails to bind GDP, suggesting that the heterodimer of these proteins constrains Ran in a conformation highly unfavorable for rebinding nucleotide. The present data indicate, therefore, that Mog1 is a nucleotide release factor but not a nucleotide exchange factor. To our knowledge, Mog1 is the first example of a GTPase regulatory protein with these properties. We also note that the specificity for binding RanGTP and the stabilization of the nucleotide-free form of Ran are features that distinguish Mog1 from RanGEF, the factor that catalyzes nucleotide exchange on Ran in the nucleus.

Mog1 was discovered by the Nishimoto laboratory (23) as a suppressor of ts alleles of Gsp1p, the S. cerevisiae orthologue of Ran. The Δmog1 strain is ts for growth and shows defects in nuclear protein import, with no obvious defect in poly(A)⁺ RNA export. S. cerevisiae Mog1p was found to bind specifically to RanGTP, however, the nucleotide release activity was not detected because the interaction was measured by protein binding. The observation that the nuclear import receptor for Ran (NTF2) can suppress the growth defect of the Δmog1 strain suggests that Mog1 could be linked to regulating nuclear levels of Ran. Thus, NTF2 and Mog1 may provide alternative mechanisms for increasing the concentration of RanGDP in the nucleus that involve nuclear import and nucleotide release, respectively. A Mog1-based mechanism for generating nuclear RanGDP would probably require an additional activity to facilitate nucleotide loading. RanGEF could fulfill this role; however, the ability of RanGEF to catalyze both GDP and GTP loading onto Ran in vivo (11–12), and the higher relative level of GTP to GDP in the cell makes this unlikely.

We speculate that despite the lack of sequence relatedness, Mog1 and RanGEF probably bind to a similar surface on Ran. This inference is based on the fact that both Mog1 and RanGEF stimulate nucleotide release from Ran, and both proteins can form a complex with Ran bound to RanBP1 (16). Indeed, RanBP1 protects Ran from nucleotide release induced by Mog1 and RanGEF. The physical basis of this protection almost certainly involves the ability of RanBP1 to stabilize the structure of the switch-I effector loop of Ran (30, 31). The switch-I effector loop in Ran, and in other Ras-like GTPases, displays a major conformational change depending on whether the bound nucleotide is GDP or GTP (30–33). We envision that, in the absence of RanBP1, Mog1 binding to Ran induces a structural change that impacts on the conformation of the switch-I effector loop, which alters the P-loop and destabilizes nucleotide binding. We note that the exchange factor Sos induces a major conformational change in the switch-I region of Ras as part of the mechanism of nucleotide release (34).

Does the ternary complex of Mog1, RanGTP, and RanBP1 identified in this study play a role in nuclear transport? RanBP1 and Ran both shuttle through the nucleus in vivo (22, 26), indicating that the three proteins could, in principle, form a complex in the nucleus. Because of the presence of a functional NES on RanBP1, the ternary complex would then be exported rapidly to the cytoplasm. In this manner, Mog1 could negatively regulate the concentration of RanGTP in the nucleus by an export-based mechanism. Further analysis of Mog1 should provide additional information about how the nucleo-
tide cycle of Ran is coupled to nuclear transport and provide new insights into the regulation of small GTPases in general.

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