Fission Yeast Mog1p Homologue, Which Interacts With the Small GTPase Ran, Is Required for Mitosis-to-Interphase Transition and poly(A)⁺ RNA Metabolism

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Manuscript received November 6, 2000
Accepted for publication January 8, 2001

ABSTRACT

We have cloned and characterized the Schizosaccharomyces pombe gene mog1⁺, which encodes a protein with homology to the Saccharomyces cerevisiae Mog1p participating in the Ran-GTPase system. The S. pombe Mog1p is predominantly localized in the nucleus. In contrast to the S. cerevisiae MOG1 gene, the S. pombe mog1⁺ gene is essential for cell viability. mog1⁺ is required for the mitosis-to-interphase transition, as the mog1-1 mutant arrests at restrictive temperatures as septated, binucleated cells with highly condensed chromosomes and an aberrant nuclear envelope. FACS analysis showed that these cells do not undergo a subsequent round of DNA replication. Surprisingly, also unlike the Δmog1 mutation in S. cerevisiae, the mog1-1 mutation causes nucleolar accumulation of poly(A)⁺ RNA at the restrictive temperature in S. pombe, but the signals do not overlap with the fibrillarin-rich region of the nucleolus. Thus, we found that mog1⁺ is required for the mitosis-to-interphase transition and a class of RNA metabolism. In our attempt to identify suppressors of mog1-1, we isolated the spi1⁺ gene, which encodes the fission yeast homologue of Ran. We found that overexpression of Spi1p rescues the S. pombe Δmog1 cells from death. On the basis of these results, we conclude that mog1⁺ is involved in the Ran-GTPase system.

THE interior of a eukaryotic cell is divided by the nuclear envelope into the nucleus (where DNA replication and RNA synthesis occur) and the cytoplasm (where protein synthesis occurs). Thus, systems for the transport of proteins and RNA across the nuclear membrane are required for cellular functions. The small GTPase Ran, which is well conserved in eukaryotes ranging from yeasts to humans, plays a key role in the nucleocytoplasmic transport of macromolecules (Gorlich 1998; Mattaj and Englmeier 1998). The functions of the Ran protein are controlled by its binding to nucleotides, which affects its ability to interact with specific target proteins (Boguski and McCormick 1993). The nucleotide-bound form of Ran is modulated by the guanine nucleotide exchange factor (GEF), which promotes the conversion of GDP to GTP, and the GTPase-activating protein (GAP), which catalyzes the hydrolysis of GTP to GDP (Dasso 1995). In nuclear protein import, proteins containing a nuclear localizing signal (NLS) form a complex with its adapter importin-α and the transporter importin-β and are then transported into the nucleus via the interaction of importin-β with nuclear pore complex (NPC). The process is completed by the release of the NLS-containing protein inside the nucleus by the attachment of GTP-bound-Ran to importin-β.

In addition to its role in nuclear protein import, Ran is also reported to play essential roles in the export of proteins and mRNA from the nucleus into the cytoplasm. In Schizosaccharomyces pombe, mutations in pim1⁺, a GEF homolog (Bischoff and Ponstingl 1991; Matsumoto and Beach 1991), result in defects in poly(A)⁺ RNA export (Kadowaki et al. 1993) and/or, possibly, in nuclear protein import (Azad et al. 1997). Similarly, in Saccharomyces cerevisiae, all temperature-sensitive (ts) alleles for the Ran homolog GSP1 have a defect in nuclear protein import, and some also exhibit defective mRNA export from the nucleus (Oki et al. 1998). Recently, MOG1 was newly isolated as a multicopy suppressor of temperature-sensitive gsp1 ts mutants in S. cerevisiae (Oki and Nishimoto 1998). Δmog1 shows temperature sensitivity and is defective in nuclear protein import but not, however, in mRNA export. Mog1p was found to bind to GTP-Gsp1p but not to GDP-Gsp1p. Recently, it was found that Mog1p functions as a guanine nucleotide release factor in vitro (Steggerda and Paschal 2000), although it remains unclear how Mog1p functions in nuclear protein import in the Ran-GTPase system.

The Ran-GTPase system is also involved in biological processes other than nucleocytoplasmic transport (Sazer...
1996). In S. cerevisiae, the mutations in the PRP20 gene, coding for GEF, also cause defects in mRNA metabolism and the nuclear structure (Aebi et al. 1990). Temperature-sensitive mutants of RNAI, which codes for Ran GAP (Becker et al., 1995), are defective in RNA processing (Hartwell 1967) as well as in nuclear protein import (Corbett et al., 1995). Finally, as shown by the pim1-d1ts mutant of S. pombe, the Ran-GTPase system is involved in cell cycle progression, especially in the mitosis-to-interphase transition. Normally, during the mitosis-to-interphase transition in yeast, the segregated chromosomes decondense, the cytoplasmic microtubule network is reorganized, and the single nuclear envelope is divided in two. The pim1-d1ts mutant, however, is arrested as septated, binucleated cells with condensed chromosomes (Sazer and Nurse 1994), and the nuclear envelope is fragmented at the restrictive temperature (Demeter et al. 1995). Interestingly, deletion or overproduction of rna1ts (encoding GAP) or sbplts (a coactivator of GAP) causes similar defects (Matynia et al. 1996; He et al. 1998), suggesting that the balance between the GDP- and GTP-bound forms of Ran is critical for cell cycle progression from mitosis to interphase.

In the present article, we isolated a S. pombe gene that encodes a protein with the potential to physically interact with the fission yeast cohesin Rad21p. Unexpectedly, the gene was found to be structurally as well as functionally homologous to the S. cerevisiae MOG1 gene, whose product interacts with the S. cerevisiae homologue of Rad21p, and was named mog1ts. Isolation and characterization of the mog1ts mutation revealed that mog1ts is required for mitosis-to-interphase transition as well as poly(A)+ RNA metabolism. In addition, we found that mog1ts genetically interacts with spi1ts, encoding the S. pombe homologue of Ran. Possible molecular functions of mog1ts in the Ran-GTPase system will be discussed.

MATERIALS AND METHODS

Yeast strains, media, and genetic methods: JY741 (h+ ade6-M216 leu1-32 ura4-D18) was used as a wild-type haploid strain of S. pombe. The wild-type diploid strain JY765 (h+/h- ade6-M216/ade6-M210 leu1-32/leu1-32 ura4-D18/ura4-D18) was used to disrupt the mog1ts gene by one-step gene disruption. YES and Edinburgh minimal medium (EMM), appropriately supplemented, were used as culturing media (Moreno et al. 1991). For transcriptional repression of Spi1p from pRep41-spi1ts, thiamine was added to EMM to yield a final concentration of 16 μM. Media containing 2% agar were used for plating. Standard genetic procedures used for S. pombe were performed as described (Gutz et al. 1974; Moreno et al. 1991).

Two-hybrid assay: For the two-hybrid screen, 3 × 106 clones of the S. pombe cDNA library in pGAD GH (Clontech, Palo Alto, CA) were screened as described in manufacturer’s instructions using the rad21ts gene as a bait in the pGBT9 plasmid. The S. cerevisiae strains CG1945 and Y190 were used as the host strains for the HisX and lacZ assays, respectively. The interaction between rad21ts and mog1ts was confirmed by both assays. The cDNA sequences in positive clones were determined with an automatic DNA sequencer (ABI PRISM 310 DNA sequencer).

Disruption of the mog1ts gene: One-step gene disruption of the mog1ts gene was carried out as previously described (Rothstein 1983). The disrupted mog1ts gene fragment, which was truncated by the insertion of the ura4ts gene, was introduced into the diploid strain JY765, generating the heterozygous diploid for mog1ts (mog1ts/ mog1::ura4ts). It was confirmed by PCR and Southern blotting that a chromosomal copy of mog1ts is correctly disrupted.

Analysis of SpMog1p-GFP localization: The plasmid expressing the S. pombe Mog1p-GFP fusion protein (pRep41-spi1ts-mog1ts-GFP) was constructed as follows. The Pds-NotI DNA fragment of pRep41-spi1ts-Ha (that carries Rep41 promoter and the spi1ts gene open reading frame (ORF)) was ligated to the Pds-NotI fragment of pGFP44 (Watanabe et al. 1997), which carries the GFP ORF, after filling both Nol termini by the Klenow fragment so that the mog1ts and GFP ORFs are fused in frame at the N and C termini, respectively. The resulting plasmid expresses the S. pombe Mog1p-GFP fusion under control of the Rep41 promoter, which is a moderate version of the nmt1 promoter (Basi et al. 1995).

Isolation of temperature-sensitive alleles of mog1ts: Isolation of temperature-sensitive alleles of mog1ts was performed as previously described (Tatebayashi et al. 1998).

Plasmid construction: For construction of the plasmids pRep41-spi1ts-mog1ts, Spi1p, and Mog1p, the ORF for each gene was amplified by PCR that altered the sequence of the initiation codon to the Ndel restriction sequence CATATG. The amplified ORFs were inserted into pRep41 plasmids such that the initiation codons were located in the Ndel site downstream of the medium nmt1 promoter (Basi et al. 1995).

Fluorescence microscopy: Cells were stained with 3,3′-dihex-yloxacarbocyanine iodide (DiOC6) to visualize the nuclear envelope (Demeter et al. 1995). Hoechst 33342 to visualize the DNA in living cells (Demeter et al. 1995), and 4′,6-diamidino-2-phenylindole (DAPI) to visualize DNA in fixed cells (Moreno et al. 1991).

FACSscan analysis: A Becton-Dickinson (San Jose, CA) FACSscan was used to estimate the DNA content by previously described procedures (Tatebayashi et al. 1998).

Analysis of nuclear protein import: Analysis of nuclear protein import was performed as previously described (Shibuya et al. 1999) with some modifications. First, the wild-type or mog1-1 strain was transformed with the plasmid pRep-NLS-GFP. This plasmid carries a DNA fragment bearing nucleoplasmin bipartite basic NLS at the N terminus and GFP at the C terminus downstream of the nmt1 promoter. The transformants were grown at 25° to early log phase in EMM without thiamine. After culturing for 1, 2, or 4 hr at 36°, the cells were washed and transferred to 10 mM sodium-azide and 10 mM 2-deoxy-D-glucose in glucose-free EMM at 36° for 1 hr. After washing, cells were resuspended and incubated in EMM at 36° for 30 min. Localization of the NLS-GFP fusion protein was determined by fluorescence microscopy.

Analysis of poly(A)+ RNA localization: Localization of poly(A)+ RNA was analyzed by in situ fluorescence hybridization (FISH) using an oligo(dT)12 probe and the D77 antibody as previously described (Tani et al. 1996).

RESULTS

The toili+ gene shows homology with MOGI: During our search for proteins that could physically interact with the fission yeast cohesin Rad21p, we cloned toili+ (rad twenty-one interacting gene) by the two-hybrid sys-
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stein 1983) by insertion with ura4 (Figure 1B). Correct integration was confirmed by PCR and Southern blotting (data not shown). After sporulation of the strain, dissection of the tetrads produced, at the most, two viable progeny at 30°C, both of which were uracil auxotrophs. Thus, the mog1+ gene appears to be essential for cell growth at 30°C, in contrast to the MOG1, which is known to be indispensable only at high temperatures (Oki and Nishimoto 1998).

A temperature-sensitive mog1 mutant (mog1-1) is defective in the mitosis-to-interphase transition: To investigate the role of mog1+ in cell growth, temperature-sensitive mog1 mutants were generated. A chromosomal copy of mog11 in haploid cells was replaced by mutated fragments of mog1 synthesized by Mn2+ PCR (Figure 2A). Two mog1 mutants that grow at 25°C but not at 35°C were isolated. The mutant that showed the most profound temperature sensitivity (mog1-1) was backcrossed with the wild-type strain and used for further analyses. Correct integration of the mutated fragment into the mog11 locus was confirmed by PCR (data not shown). The temperature sensitivity of mog1-1 could be abrogated by introducing the mog11-containing plasmid (Figure 2B), and mog1-1/mog11 heterozygous diploid cells were not

Figure 1.—(A) Comparison of amino acid sequences of SpMog1p and ScMog1p. Amino acid sequences are aligned between SpMog1p and ScMog1p. Double dots indicate conserved residues, and single dots represent similar residues. Four amino acid substitutions identified in the mog1-1 allele are also shown. Amino acids S151 (TCC), S152 (TCA), V153 (GTT), and L185 (CTT) were changed to C (TGC), T (ACA), A (GCT), and R (CGT), respectively, in the mog1-1 allele. Underlines show the mutated nucleotides in mog1-1. (B) The construct with a disruption of the mog11 gene. Restriction sites are shown at the top of the mog11 locus. The arrow indicates the ORF for the mog11 gene and the direction of transcription. S, SalI; T22I, EcoT22I. The construct of the plasmid for disruption of the toi11 gene is shown at the bottom of the figure. The selection marker ura4 was inserted into the EoTI22I site within the ORF for the mog11.

tem. The cDNA clone was sequenced and found to be identical to the uncharacterized ORF SPCC1840.01c that encodes a 191-amino-acid protein (EMBL accession no. AL031179) in the S. pombe chromosome III cosmid c1840. A database search for homology revealed that toi11 has significant homology with the S. cerevisiae MOG1 gene (30.7% identity in 199 overlapping amino acids; Figure 1A), and toi11 was renamed mog1+. MOG1 was shown to be able to suppress the temperature sensitivity of S. cerevisiae strains with mutations in the Ran-homolog gene GSP1 (Oki and Nishimoto 1998).

The mog1+ gene is involved in cell proliferation: To determine whether mog1+ is required for cell proliferation, one of the mog1+ genes in the diploid yeast strain JY765 was disrupted by one-step gene disruption (Roth-
affected by temperature (data not shown), indicating that the mog1-1 mutation is recessive. The temperature sensitivity of mog1-1 was also suppressed by expression of S. cerevisiae MOG1 (Figure 2B). Thus, mog1+ is not only structurally but functionally homologous to MOG1. Sequencing of the mog1-1 allele revealed that four amino acid residues (S151, S152, V153, and L185) located in the C-terminal region were substituted with cysteine, threonine, alanine, and arginine, respectively, in mog1-1 (Figure 1B). Either or both of two regions (the regions around 151–153 and 185) may be required for functions of the S. pombe Mog1p at high temperature.

Nuclear staining of mog1-1 cells indicated that these cells had some interesting features. After culturing the mog1-1 cells at the restrictive temperature of 36°, the population of the binucleated cells with condensed chromosomes and a septum dramatically increased (Figure 3, A and B). This population constituted ~70% of the cells when cultured for 5 hr at the restrictive temperature, after which mononucleated cells with condensed chromosomes began to appear. In contrast, only 10% of wild-type cells had such features (data not shown). FACS analysis revealed that the majority of the mog1-1 cells at the restrictive temperature contained a 2C DNA content (Figure 3C), suggesting that the septated, binucleated cells had a 1C DNA content per nucleus. This indicates that these cells did not proceed to the next round of DNA replication. The minor peak of 1C seen after 5 hr incubation might represent the mononucleated cells with condensed chromosomes, which had possibly been derived from the arrested, binucleated cells but had not proceeded to the next round of replication. These results together suggest that the mog1-1 mutant is defective in the mitosis-to-interphase transition. In addition, when stained with DiOC6 to visualize the nuclear envelope, the mog1-1 cells cultured at the restrictive temperature did not exhibit the characteristic outline of the nucleus (Figure 3A), indicating that the structure of the nuclear envelope may also be aberrant in mog1-1.

The S. pombe Mog1p is localized in the nucleus: To assess where the S. pombe Mog1p (SpMog1p) is localized in the cell, the plasmid pRep41-mog1-GFP, whose expression of the SpMog1p-GFP fusion protein is induced

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**Figure 3.**—Analysis of nuclear morphology and DNA content of the mog1-1 mutant. (A) DNA and nuclear envelope structures of the mog1-1 mutant. mog1-1 cells grown at 36° for 5 hr or at 25° were stained with Hoechst to visualize the nucleus and with DiOC6 to visualize the nuclear envelope. (B) Frequency of the cells defective in mitosis-to-interphase transition in the mog1-1 mutant. mog1-1 cells grown at 25° were transferred to 36° and cells were collected, fixed, and stained with DAPI. The frequency of each cell type is indicated. Solid circles indicate the interphase chromatin; solid squares, binucleated cells with highly condensed chromosomes and a septum; solid triangles, mononucleate cells with highly condensed chromosomes. At least 100 cells were counted for each sample. (C) FACScan analysis of the mog1-1 mutant grown at 36°. Cells grown at 25° were then cultured at 36°, and aliquots were collected for DNA content analysis by a Becton-Dickinson FACScan. The DNA content and relative cell number were plotted along with x- and y-axes, respectively. The positions of 1C, 2C, and 4C DNA content are indicated. Left, mog1-1 cells; right, wild-type (WT) cells.
in the thiamine-free medium, was constructed. The introduction of this plasmid completely abrogated the temperature sensitivity of the mog1-1 mutant, even on the thiamine-containing plates (data not shown), thus indicating that the SpMog1p-GFP fusion protein is functional. When the mog1-1 cells containing the plasmid were grown in thiamine-free EMM medium, the GFP signal was detected predominantly, but not exclusively, in the nucleus (Figure 4). However, when SpMog1p-GFP expression was largely repressed by the presence of thiamine, no significant GFP signals could be seen (data not shown).

**Nuclear protein import in the mog1-1 mutant:** In fission yeast, the Ran-GTPase system is known to be required for the mitosis-to-interphase (Sazer and Nurse 1994; Matynia et al. 1996; He et al. 1998) as well as for nuclear protein import and mRNA export (Kadowaki et al. 1993; Shibuya et al. 1999). As mog1+ was found to be involved in the mitosis-to-interphase, we speculated that mog1+ could be involved in the Ran-GTPase system and thus may also participate in nucleocytoplasmic macromolecule transport. This possibility was assessed by examining the localization of a nuclear protein (represented by the GFP-NLS fusion protein) in the mog1-1 mutant. The mog1-1 mutant and the wild-type cells were both transformed with the pRep-GFP-NLS plasmid (Shibuya et al. 1999) and then grown at 25°C in EMM medium. In these conditions, the GFP-NLS protein is expressed and localized to the nucleus. After preincubation for 1, 2, or 4 hr at the restrictive temperature of 36°C, cells were treated with azide and deoxyglucose for 1 hr at 36°C, which results in the poisoning of the energy metabolism and the diffusion of the NLS-GFP fusion protein into the cytoplasm. After release from drug treatment into glucose-containing EMM, the NLS-GFP fusion protein was allowed to relocalize to the nucleus at 36°C for 30 min, after which the cells were assessed by fluorescence microscopy. When the cells were preincubated at 36°C for 1 or 2 hr, the NLS-GFP fusion protein was found to have been reimported into the nucleus in the mog1-1 mutant as well as into the wild-type cells (data not shown). However, when preincubated at 36°C for 4 hr, the NLS-GFP fusion protein remained diffused in most of the septated cells of mog1-1, whereas reimport of the NLS-GFP fusion protein was not inhibited in the wild-type cells (Figure 5). Thus, the mog1+ gene product appeared to be involved in nuclear protein import. However, because the nuclear envelope becomes abnormal in the mog1-1 cells after prolonged incubation at the restrictive temperature, it is likely that the apparent defect of mog1-1 in nuclear protein import may be caused by the loss of nuclear envelope integrity.

**Poly(A)+ RNA accumulates in the nucleolus of the mog1-1 cells:** To investigate the effect of mog1-1 on mRNA export from the nucleus, FISH was used to analyze the localization of poly(A)+ RNA in the cells. The
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Figure 6.—Analysis of poly(A)+ RNA localization in the wild-type or the mog1-1 cells. Wild-type or mog1-1 cells grown at 26° were cultured at 37° for 2 hr. The cells were fixed and subjected to triple staining with the biotin-labeled oligo(dT) probe [poly(A)+ RNA], the D77 antibody (fibrillarin), and DAPI (DNA). On the right are merged images.

mog1-1 cells grown at 26° were cultured at 37° and then subjected to triple staining with an oligo(dT) probe that anneals to the poly(A) tail of RNA, the D77 antibody that specifically recognizes a nucleolar protein, fibrillarin, and DAPI, which stains DNA (Figure 6). Poly(A)+ RNA was found to be distributed throughout the cells in the wild-type cells at both 26° and 37°. A similar pattern was observed for the mog1-1 cells grown at 26°. After culture at 37°, however, focal accumulations of poly(A)+ RNA were observed in the mog1-1 cells. The RNA signals accumulated outside of the DNA region, which corresponds to the nucleolus region. However, the accumulated RNA did not colocalize with fibrillarin, and the fibrillarin-rich region appeared to become smaller in the mog1-1 cells at 37°.

Figure 7 shows the kinetics of poly(A)+ RNA accumulation in the mog1-1 cells at the restrictive temperature. Cells with nucleolar RNA signals were first observed after ~30 min at 37°. Their numbers peaked after 2 hr (~80%), when most of the cells had a normal nuclear envelope (data not shown), and then decreased to ~20% after 4 hr, becoming negligible after 6 hr. Interestingly, the intensity of the cytoplasmic poly(A)+ RNA signal did not significantly decrease regardless of the degree of nucleolar accumulation of poly(A)+ RNA (Figures 6 and 7), suggesting that most mRNAs continue to be exported to the cytoplasm at the restrictive temperature. Thus, in the mog1-1 mutant, export of particular mRNA species might be inhibited, resulting in the accumulation of these RNAs in the nucleolus. Alternatively, polyadenylated small nucleolar RNAs may accumulate abnormally at the restrictive temperature as has been reported for some exosome mutants (Kadowaki et al. 1994; van Hoof et al. 2000; see discussion).

The temperature sensitivity of the mog1-1 mutant and the lethality of the mog1 null mutant are both suppressed by overexpression of spi1+ coding for Ran homologue:

To assess the molecular functions of mog1+, high-copy suppressors of the mog1-1 mutation were isolated. Thus, the fission yeast genomic and cDNA libraries were introduced into mog1-1, and clones lacking the temperature sensitivity of mog1-1 were identified (Figure 8A). Sequence analysis showed that the cDNA clones isolated in this way contain the spi1+ gene, which encodes the Ran-GTPase in fission yeast (Matsumoto and Beach 1991). Among the four genomic clones isolated, two were confirmed to contain the spi1+ gene by PCR. To exclude the possibility that a gene adjacent to the spi1+ gene may be involved in the suppression of temperature sensitivity, the plasmid containing the selection marker LEU2 and the ORF for spi1+ under the control of Rep41 promoter (pRep41-spi1+) was constructed and introduced into the mog1-1 mutant. The mog1-1 cells carrying pRep41-spi1+ grew at the restrictive temperature (data not shown), indicating that overexpression of Spi1p alone can overcome the temperature sensitivity of the mog1-1 mutant.

It was also assessed whether Spi1p overexpression can rescue the S. pombe Δmog1 cells from death. After sporulation of heterozygous mog1+/mog1::ura4+ diploid cells harboring the LEU2-containing plasmid pRep41-spi1+, the Ura+ and Leu+ progeny were selected on EMM plates. Cells expressing Spi1p were viable (Figure 8B). When Spi1p expression was shut off, the mog1::ura4+ cells with pRep41-spi1+ ceased to grow (Figure 8B), and most cells were arrested as a binucleate cell with highly
condensed chromosomes (Figure 8C). Thus, mog1-1 is a loss-of-function mutation of mog1+, and high doses of Spi1p compensate for the lack of mog1+ function.

DISCUSSION

In this study, a novel S. pombe gene, named mog1+, was isolated and the function of its gene product was characterized. Sequencing of the gene revealed that the gene encodes a protein that has 30.7% homology to the S. cerevisiae MOG1 gene product. It is interesting that mog1+ is required for cell growth, although MOG1 is dispensable for cell viability except at high temperatures. To assess whether the functions that mog1+ plays in cell proliferation are distinct from those played by MOG1, the effect of the S. cerevisiae Mog1p (ScMog1p) overexpression was examined in the temperature-sensitive mog1-1 mutant. Because overexpression of ScMog1p abrogated the temperature sensitivity of mog1-1, it was concluded that the functions of ScMog1p and SpMog1p overlap. Thus, mog1+ is not only structurally but also functionally homologous to MOG1. Although the mog1+ gene was originally identified by the two-hybrid assay on the basis of its putative interaction with Rad21p, the mutation sites in the mog1-1 allele revealed that the Mog1p (ScMog1p) structure of ScMog1p, the region containing aminois acids 151–153 forms an α-helix, while the other region forms a β-sheet (Stewart and Baker 2000). Mog1p homologues were found in human, mouse, and Caenorhabditis elegans as well as in S. pombe by sequence database searches. Four amino acid residues substituted into the mog1-1 allele are considerably conserved among the...
species (Stewart and Baker 2000), suggesting that they may be required for efficient functions of SpMog1p at high temperature. It will be necessary to identify the mutation(s) responsible for temperature sensitivity of mog1-1.

Analysis of the mog1-1 mutant also uncovered other features in common with MOG1. The mog1-1 gene genetically interacts with the spi1+ gene, which encodes the Ran homologue in S. pombe (Matsumoto and Beach 1991), as does MOG1 with GSP1 in S. cerevisiae. MOG1 was originally isolated as a multicopy suppressor of the gsp1 mutant, and ScMog1p binds to GTP-Gsp1p but not GDP-Gsp1p (Oki and Nishimoto 1998). In our screening for multicopy suppressors of the mog1-1 mutation, an increased dosage of Spi1p was found to overcome the temperature sensitivity of the mog1-1 mutant in growth, and, moreover, it rescued the S. pombe Δmog1 mutant cells from death. These results indicate that SpMog1p may directly or indirectly regulate the function of Spi1p. Spi1p exists predominantly in the nucleus (Matyňa et al. 1996), and the nuclear Spi1p generally exists in a GTP-bound form. SpMog1p, which also localizes in the nucleus when overexpressed, is thus likely to control the function of Spi1p through its direct interaction with GTP-Ran in the nucleus.

While SpMog1p and ScMog1p share some functions, SpMog1p also appears to have functions distinct from those of ScMog1p. While nuclear protein import was severely blocked in the S. cerevisiae Δmog1 mutant, distribution of poly(A)+ RNA was not affected at all (Oki and Nishimoto 1998), suggesting that ScMog1p is not involved in mRNA export. In contrast, the nucleoli of the mog1-1 cells exhibit intense poly(A)+ RNA signals at the restrictive temperature. However, the signals do not overlap with the fibrillarin-rich region of the nucleolus. Since the intensity of cytoplasmic signals did not appear to be reduced at the restrictive temperature, global export of mRNA should not be impaired but transport of specific mRNA species may be inhibited. Alternatively, the nucleolar signals present at the restrictive temperature may represent polyadenylated small nucleolar RNAs (snoRNAs). In S. cerevisiae, some snoRNAs are known to be polyadenylated and then processed by the exosome. In some exosome mutants, the deadenylation of poly(A)+ tails is impaired and this results in increased levels of polyadenylated snoRNA species (van Hoof et al. 2000). The budding yeast mtr3-1 and mtr4-1 mutants were originally isolated as mutants that accumulated poly(A)+ RNA in the nucleolus and were possibly defective in mRNA export (Kadowaki et al. 1994). The mtr3-1 and mtr4-1 mutants carry a mutation in the gene encoding the exosome component and further study has revealed that the level of polyadenylated snoRNAs is increased in each mutant (van Hoof et al. 2000). Thus, the poly(A)+ RNA accumulating in the nucleolus of the mtr3-1 and mtr4-1 mutants is most likely polyadenylated snoRNAs whose poly(A)+ tails should have been removed by the exosome. Interestingly, Dis3p, a component of the exosome (Mitchell et al. 1997), is known to physically interact with Ran and enhance the GEF activity of the RCC1 homologue (Noguchi et al. 1996). In S. pombe, nucleolar accumulation of poly(A)+ RNA has also been observed in the strain with a mutation in the RCC1 homologue (pim1/pts2; Azad et al. 1997). Our data on SpMog1p could thus also suggest that this protein may be directly or indirectly involved in processing of polyadenylated snoRNAs in collaboration with Spi1p. In the mog1-1 mutant, the accumulated signals of RNA in the nucleolus do not colocalize with the nucleolar protein fibrillarin, which is known to bind several snoRNA species. It is possible that aberrantly polyadenylated snoRNAs may be dissociated from fibrillarin in the mog1-1 mutant. Unlike the mog1-1 mutant, the accumulated RNA signals in the nucleolus overlap with the fibrillarin-rich region in the pim1 mutant (Azad et al. 1997), suggesting that SpMog1p functions in the Ran-GTPase system for RNA metabolism in a different manner from the GEF of Spi1p. However, regardless of which hypothesis is true, it is clear that SpMog1p is involved in RNA metabolism. The RNA species that accumulate in mog1-1 cell nucleoli must be identified before we can determine the exact role of SpMog1p in RNA metabolism.

As for nuclear protein import, we could not conclude that SpMog1p is primarily involved in it like ScMog1p. In the S. cerevisiae Δmog1 mutant, nuclear import of the reporter protein (bearing either the classical NLS of H2B or the nonclassical NLS-containing Npl3p) is inhibited at high temperatures (Oki and Nishimoto 1998). In the mog1-1 mutant, reimport into the nucleus of the GFP reporter protein bearing the nucleolus basic NLS is not significantly blocked when preincubated for 1 or 2 hr at the restrictive temperature. When most of the mog1-1 cells become septated ones with an aberrant nuclear envelope, nuclear protein import appears to be blocked at the restrictive temperature. Therefore, it is likely that mis-localization of a nuclear receptor to the cytoplasm might be due to the absence of the nuclear envelope. Perhaps we should examine the integrity of nuclear envelopes by more specific visualization of them using the antibody against nuclear pore complex or, additionally, by thin-section electron microscopy.

Another important role of SpMog1p not shared by ScMog1p is in regulation of cell cycle progression, especially in mitosis-to-interphase transition. The mog1-1 mutant exhibited a defect in mitosis-to-interphase transition, where most of the cells were arrested before the initiation of S phase as a binucleated, septated cell with highly condensed chromosomes. Furthermore, the structure of the nuclear envelope was found to be aberrant in the mutant cells. A defect in mitosis-to-interphase transition has been previously reported in several S. pombe mutants that have defects in the Ran-GTPase sys-
tum. These include the *pim1-d1* mutant (which is mutated in the *pim1* gene encoding the GEF of Spi1p; Sazer and Nurse 1994) and mutants depleted in or overexpressing Rna1p (GAP) or Sbp1p (a coactivator of GAP; Matynia et al. 1996; He et al. 1998). In these situations, an imbalance between the GTP and GDP form of Spi1p is presumed to cause this phenotype. That the *mog1* and *spt1* genes interact genetically also suggests that the defective transition from mitosis to interphase in the *mog1* mutant is caused by perturbation of the Ran-GTPase system. However, it is not clear why this defect in the Ran-GTPase system results in cell cycle arrest at the mitosis-to-interphase transition.

How, then, is SpMog1p involved in the Ran-GTPase system? In budding yeast, the temperature sensitivity of ∆*mog1* is suppressed by overproduction of Ntf2p as well as Gsp1p (Oku and Nishimoto 1998). Recent studies have revealed that Ntf2p attaches to GDP-Ran and imports it from the cytoplasm to the nucleus (Ribbeck et al. 1998; Smith et al. 1998), thus maintaining a high concentration of Ran in the nucleus. This suggests that Spi1p overexpression might contribute to the suppression of the *mog1* mutant, at the temperature sensitivity of the *pim1* mutants, in which the conversion to the GTP form of Spi1p in the nucleus is inhibited, is also abrogated by an increased dosage of Spi1p (Matsumoto and Beach 1991; Sazer and Nurse 1994). Recently, it was found that murine Mog1p functions as a guanine nucleotide release factor in vitro (Steggerda and Paschal 2000). It is possible that SpMog1p may regulate the nucleotide-bound state of Spi1p by the guanine nucleotide release activity. Biochemical studies of SpMog1p are required to test this possibility. Alternatively, or in addition, the *mog1* mutant may have lost the ability to maintain a high concentration of Spi1p in the nucleus. To address the possibility, we have tried to determine whether the mutation in *mog1* affects the nuclear accumulation of Spi1p. When the Spi1-GFP fusion protein was expressed in the *mog1* mutant, it was found to be predominantly located in the nucleus at the permissive temperature (K. Tatebayashi and H. Ikeda, unpublished results), as seen in wild-type cells (Matynia et al. 1996). The shift of the temperature to 36°C did not affect the location of Spi1p-GFP (K. Tatebayashi and H. Ikeda, unpublished results), suggesting that SpMog1p is unlikely to be essential for the nuclear accumulation of Spi1p. However, as the fusion protein did not abrogate the temperature sensitivity of the *mog1* mutant, it may be necessary to assess the localization of intrinsic Spi1p before any firm conclusions can be drawn.

We are grateful to Dr. V. Watanabe and Dr. T. Takeda for providing plasmids and libraries, Dr. J. P. Aris for providing the D77 antibody, and E. Hirose for technical assistance. This work was supported by a grant from the Ministry of Education, Science, Sports and Culture of Japan.

Note added in proof: In addition to four amino acid substitutions described in the text, isoleucine at the position of 160 was also substituted with threonine in *mog1-1*.

**LITERATURE CITED**


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Communicating editor: F. Winston