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

K. Tatebayashi,* T. Tani[†,‡] and H. Ikeda*[,§]

**Department of Molecular Biology, Institute of Medical Science, the University of Tokyo, P.O. Takanawa, Tokyo 108-8639, Japan, [†]Department of Biology, Graduate School of Science, Kyushu University, Fukuoka 812-8581, Japan, [‡]PRESTO, Japan Science and Technology Corporation, Fukuoka 812-8581, Japan and [§]Microbial Chemistry, Center for Basic Research, The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan*

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 Δ*mog1* 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* Δ*mog1* 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* mutants in *S. cerevisiae* (Oki and Nishimoto 1998). Δ*mog1* 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

Corresponding author: Kazuo Tatebayashi, Department of Molecular Biology, Institute of Medical Science, the University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail: tategone@ims.u-tokyo.ac.jp

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 *RNA1*, 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-d1*^{ts} 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-d1*^{ts} 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, depletion or overproduction of *rna1*$^+$ (encoding GAP) or *sbp1*$^+$ (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 Ran, and was named *mog1*$^+$. Isolation and characterization of the *mog1*^{ts} mutant revealed that *mog1*$^+$ is required for mitosis-to-interphase transition as well as poly(A)$^+$ RNA metabolism. In addition, we found that *mog1*$^+$ genetically interacts with *spi1*$^+$, encoding the *S. pombe* homologue of Ran. Possible molecular functions of *mog1*$^+$ 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 *mog1*$^+$ 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-*spi1*$^+$, 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, 3×10^6 clones of the *S. pombe* cDNA library in pGAD GH (Clontech, Palo Alto, CA) were screened as described in manufacturer's instructions using the *rad21*$^+$ gene as a bait in the pGBT9 plasmid. The *S. cerevisiae* strains CG1945 and Y190 were used as the host strains for the His$^+$ and lacZ assays, respectively. The interaction between *rad21*$^+$ and *mog1*$^+$ 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 *mog1*$^+$ gene: One-step gene disruption of the *mog1*$^+$ gene was carried out as previously described (Rothstein 1983). The disrupted *mog1*$^+$ gene fragment, which was truncated by the insertion of the *ura4*$^+$ gene, was introduced into the diploid strain JY765, generating the heterozygous diploid for *mog1*$^+$ (*mog1*$^+$/*mog1::ura4*$^+$). It was confirmed by PCR and Southern blotting that a chromosomal copy of *mog1*$^+$ is correctly disrupted.

Analysis of SpMog1p-GFP localization: The plasmid expressing the *S. pombe* Mog1p-GFP fusion protein (pRep41-*mog1*$^+$-GFP) was constructed as follows. The *Pst*I-*Not*I DNA fragment of pRep41-*mog1*$^+$-Ha [that carries Rep41 promoter and the *mog1*$^+$ gene open reading frame (ORF)] was ligated to the *Pst*I-*Not*I fragment of pGFT41 (Watanabe *et al.* 1997), which carries the GFP ORF, after filling both *Not*I termini by the Klenow fragment so that the *mog1*$^+$ 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.* 1993).

Isolation of temperature-sensitive alleles of *mog1*: Isolation of temperature-sensitive alleles of *mog1* was performed as previously described (Tatebayashi *et al.* 1998).

Plasmid construction: For construction of the plasmids pRep41-*mog1*$^+$, *spi1*$^+$, and *MOG1*, the ORF for each gene was amplified by PCR that altered the sequence of the initiation codon to the *Nde*I restriction sequence CATATG. The amplified ORFs were inserted into pRep41 plasmids such that the initiation codons were located in the *Nde*I site downstream of the medium *nmt1* promoter (Basi *et al.* 1993).

Fluorescence microscopy: Cells were stained with 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$) 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).

FACScan analysis: A Becton-Dickinson (San Jose, CA) FACScan 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)$_{50}$ probe and the D77 antibody as previously described (Tani *et al.* 1996).

RESULTS

The *toi1*$^+$ gene shows homology with *MOG1*: During our search for proteins that could physically interact with the fission yeast cohesin Rad21p, we cloned *toi1*$^+$ (rad *t*wenty-*o*ne *i*nteracting gene) by the two-hybrid sys-

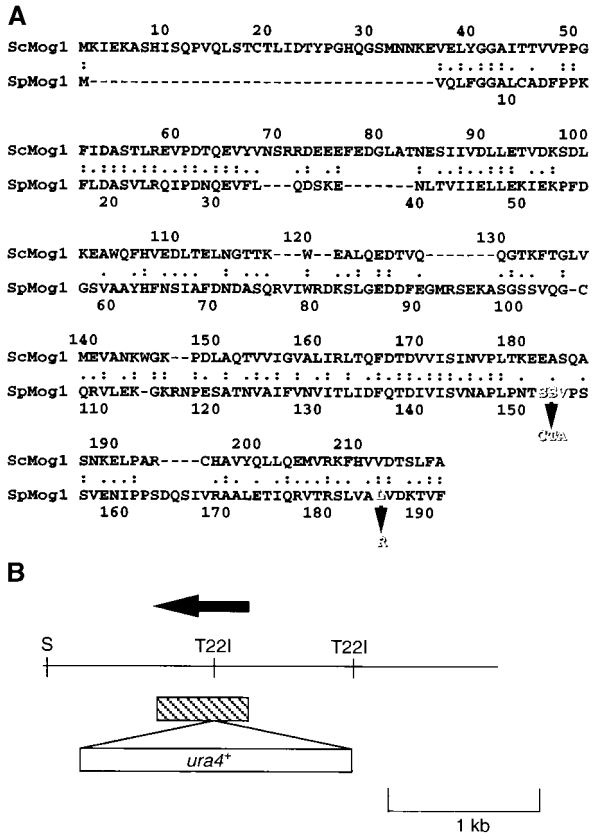

FIGURE 1.—(A) Comparison of amino acid sequences of SpMog1p and ScMog1p. Amino acid sequences are aligned between SpMog1p and ScMog1p. Double dots indicate conserved residues, and single dots represent similar residues. Four amino acid substitutions identified in the *mog1-1* allele are also shown. Amino 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 *mog1*$^+$ gene. Restriction sites are shown at the top of the *mog1*$^+$ locus. The arrow indicates the ORF for the *mog1*$^+$ gene and the direction of transcription. S, *Sal*I; T22I, *Eco*T22I. The construct of the plasmid for disruption of the *toi1*$^+$ gene is shown at the bottom of the figure. The selection marker *ura4*$^+$ was inserted into the *Eco*T22I site within the ORF for the *mog1*$^+$.

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 *toi1*$^+$ has significant homology with the *S. cerevisiae MOG1* gene (30.7% identity in 199 overlapping amino acids; Figure 1A), and *toi1*$^+$ 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 (ROTHSTEIN 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°, both of which were uracil auxotrophs. Thus, the *mog1*$^+$ gene appears to be essential for cell growth at 30°, 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 *mog1*$^+$ in haploid cells was replaced by mutated fragments of *mog1* synthesized by Mn^{2+} PCR (Figure 2A). Two *mog1* mutants that grow at 25° but not at 35° 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 *mog1*$^+$ locus was confirmed by PCR (data not shown). The temperature sensitivity of *mog1-1* could be abrogated by introducing the *mog1*$^+$-containing plasmid (Figure 2B), and *mog1-1/mog1*$^+$ heterozygous diploid cells were not

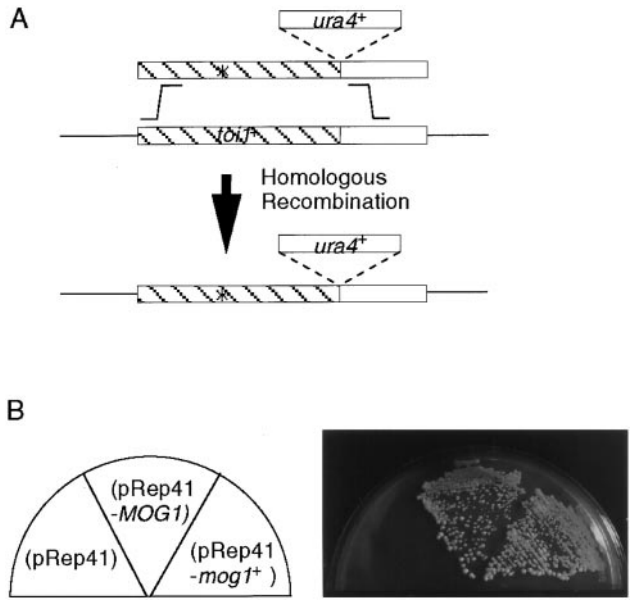

FIGURE 2.—Isolation of temperature-sensitive *mog1* mutations in fission yeast and their rescue by plasmids expressing *S. pombe mog1*$^+$ or *S. cerevisiae MOG1*. (A) Scheme for isolation of *mog1*^{ts} mutants in fission yeast. A pool of mutated *mog1* DNA fragments with the selection marker *ura4*$^+$ gene was introduced into the Δ*ura4* strain. The chromosomal *mog1*$^+$ gene was then replaced by the mutated one through homologous recombination. *ura4*$^+$ was inserted between the *mog1*$^+$ open reading frame (striped box) and its downstream region (open box). An asterisk (*) indicates the postulated mutation site(s). (B) Suppression of *mog1-1*^{ts} by overexpression of *mog1* or *MOG1*$^+$. The *mog1-1* strain was transformed with pRep41, pRep41-*mog1*$^+$, or pRep41-*MOG1*, and the transformants were streaked onto EMM at 35°.

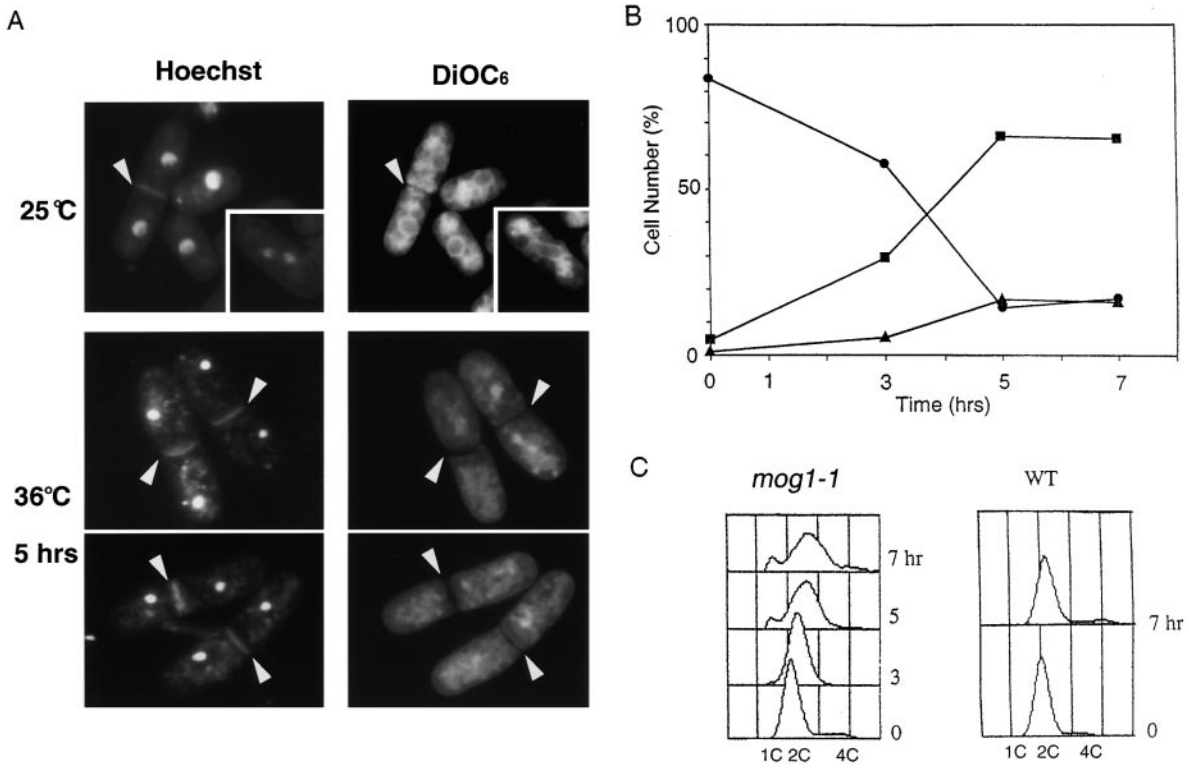

FIGURE 3.—Analysis of nuclear morphology and DNA content of the *mog1-1* mutant. (A) DNA and nuclear envelope structures of the *mog1-1* mutant. *mog1-1* cells grown at 36° for 5 hr or at 25° were stained with Hoechst to visualize the nucleus and with $DiOC_6$ to visualize the nuclear envelope. (B) Frequency 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.

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 $DiOC_6$ 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

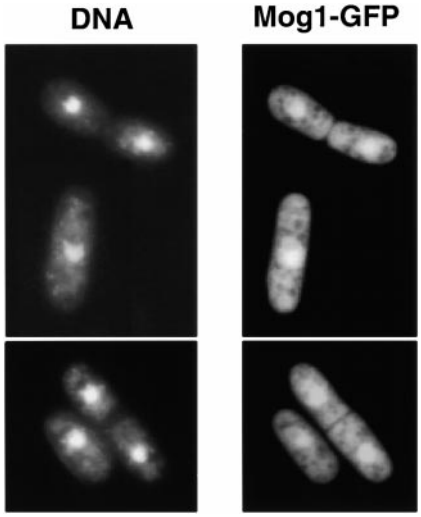

FIGURE 4.—Intracellular localization of SpMog1p. *mog1-1* cells transformed with the plasmid containing the *mog1*$^+$-GFP gene were analyzed by fluorescence microscopy. DNA shows cells stained with DAPI in the corresponding field.

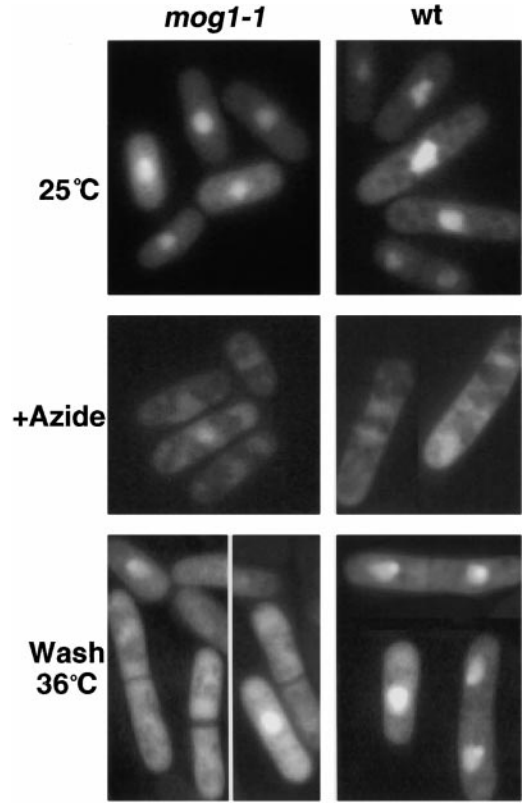

FIGURE 5.—Analysis of nuclear protein import. *mog1-1* and wild-type (wt) cells expressing the NLS-GFP fusion protein were grown at 25° (top). Cells were preincubated for 4 hr at 36° and then metabolically poisoned to allow diffusion of the NLS-GFP fusion protein in cells treated with azide and deoxyglucose for 1 hr (middle). After recovery from the drug treatment in glucose-containing EMM medium for 30 min at 36°, reimport of the NLS-GFP fusion protein into the nucleus was analyzed (bottom). The NLS-GFP fusion protein was visualized by fluorescence microscopy.

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 RanGTPase 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° 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°, cells were treated with azide and deoxyglucose for 1 hr at 36°, 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° for 30 min, after which the cells were assessed by fluorescence microscopy. When the cells were preincubated at 36° 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° 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

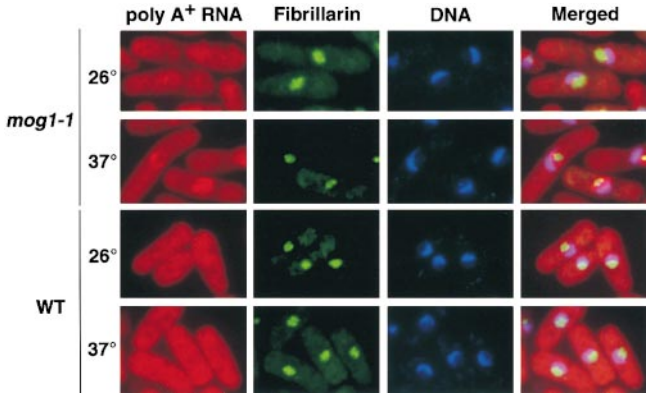

FIGURE 6.—Analysis of poly(A)$^+$ RNA localization in the wild-type or the *mog1-1* cells. Wild-type or *mog1-1* cells grown at 26° were cultured at 37° for 2 hr. The cells were fixed and subjected to triple staining with the biotin-labeled oligo(dT)$_{50}$ probe [poly(A)$^+$ RNA], the D77 antibody (fibrillarin), and DAPI (DNA). On the right are merged images.

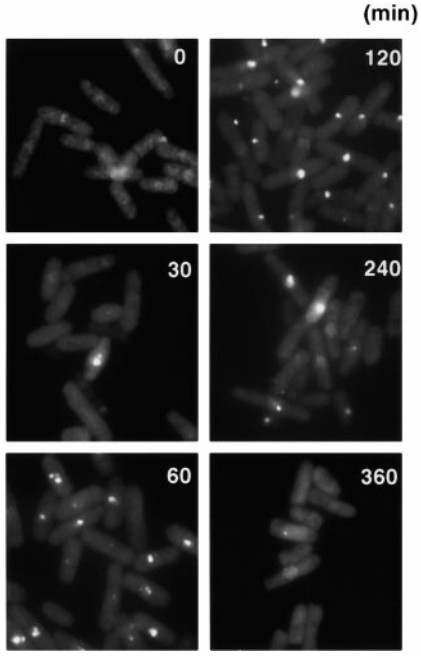

FIGURE 7.—Rapid accumulation of poly(A)$^+$ RNA after shift up to the restrictive temperature in the *mog1-1* cells. The *mog1-1* cells grown at 26° were cultured at 37° at the indicated times and subjected to FISH with a biotin-labeled oligo(dT)$_{50}$ probe. Hybridized signals were detected by FITC-conjugated avidin.

mog1-1 cells grown at 26° were cultured at 37° and then subjected to triple staining with an oligo(dT)$_{50}$ 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 abberantly 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* Δ*mog1* 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

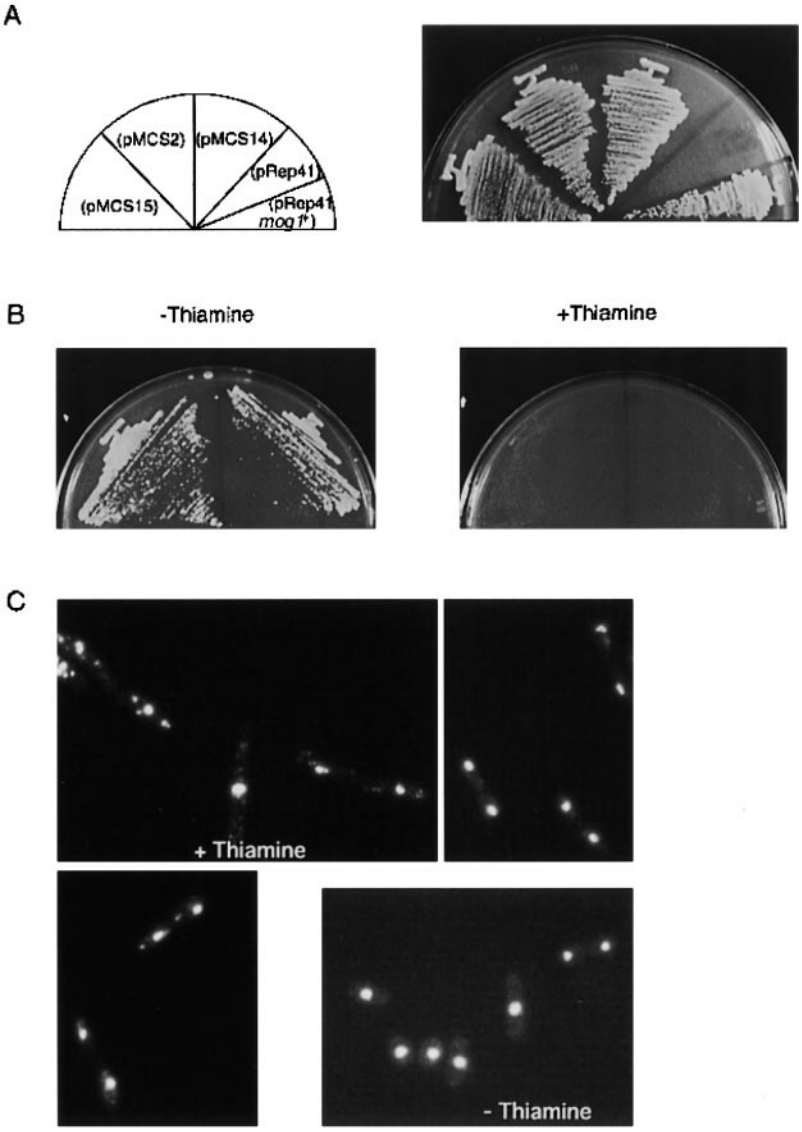

FIGURE 8.—Suppression of the *S. pombe mog1* mutants by overexpression of the *spi1*$^+$ gene. (A) Suppression of *mog1-1*^{ts} by overexpression of *spi1*$^+$. The *mog1-1* strain was transformed with pRep41, pRep41-*mog1*$^+$, pMCS15, pMCS2, or pMCS14, and they were streaked onto EMM at 35°. pMCS15, pMCS2, and pMCS14 were isolated as multicopy suppressors of *mog1-1*^{ts}. pMCS15 and pMCS2 contain the *spi1*$^+$ genomic clone, and pMCS14 contains the *spi1*$^+$ cDNA clone. (B) Suppression of the *S. pombe* Δ*mog1* mutant by overexpression of *spi1*$^+$. The *mog1::ura4*$^+$ cells with pRep41-*spi1*$^+$ were streaked onto EMM with or without thiamine at 30°. (C) Nuclear morphology of the *S. pombe* Δ*mog1* mutant after shutting off plasmid expression of *spi1*$^+$. The Δ*mog1* cells harboring pRep41-*spi1*$^+$ grown in thiamine-free medium were transferred to thiamine-containing medium. After 20 hr incubation, cells were collected, fixed, stained by DAPI, and analyzed by fluorescence microscopy.

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, a fission yeast cohesin (BIRKENBIHL and SUBRAMANI 1992; GUACCI *et al.* 1997; MICHAELIS *et al.* 1997; TATEBAYASHI *et al.* 1998), the relationship between SpMog1p and the cohesin Rad21p is at present unclear. Determination of the mutation sites in the *mog1-1* allele revealed that the mutations reside in two C-terminal regions around amino acids 151–153 and 185. Judging from the crystal structure of ScMog1p, the region containing amino 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 in 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*$^+$ 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-Gsp1 (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* Δ*mog1* mutant cells from death. These results indicate that SpMog1p may directly or indirectly regulate the function of Spi1p. Spi1p exists predominantly in the nucleus (Matynia *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* Δ*mog1* 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/ptr2*; 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* Δ*mog1* 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 nucleoplasmin bipartite 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-

tem. 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 *spi1*$^+$ genes interact genetically also suggests that the defective transition from mitosis to interphase in the *mog1-1* 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 Δ*mog1* is suppressed by overproduction of Ntf2p as well as Gsp1p (Oki 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 *mog1-1* phenotype at high temperatures by increasing the level of Spi1p in the nucleus. Such enrichment of Spi1p in the nucleus might overcome a reduced activity or a decreased protein level of nuclear Spi1p caused by the *mog1-1* mutation. With regard to the former possibility, perturbations in the balance between the GTP and GDP forms of Spi1p might occur in the *mog1-1* mutant, as 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 *mog1-1* 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 *mog1-1* 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° 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 *mog1-1* mutant, it may be necessary to assess the localization of intrinsic Spi1p before any firm conclusions can be drawn.

We are grateful to Dr. Y. Watanabe and Dr. T. Takeda for providing plasmids and libraries, Dr. J. P. Aris for providing the D77 antibody, and E. Hirosawa 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

Aebi, M., M. W. Clark, U. Vijayraghavan and J. Abelson, 1990 A yeast mutant, PRP20, altered in mRNA metabolism and maintenance of the nuclear structure, is defective in a gene homologous to the human gene RCC1 which is involved in the control of chromosome condensation. Mol. Gen. Genet. **224:** 72–80.

Azad, A. K., T. Tani, N. Shiki, S. Tsuneyoshi, S. Urushiyama *et al.*, 1997 Isolation and molecular characterization of mRNA transport mutants in *Schizosaccharomyces pombe*. Mol. Biol. Cell **8:** 825–841.

Basi, G., E. Schmid and K. Maundrell, 1993 TATA box mutations in the *Schizosaccharomyces pombe nmt1* promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. Gene **123:** 131–136.

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

Birkenbihl, R. P., and S. Subramani, 1992 Cloning and characterization of *rad21* an essential gene of *Schizosaccharomyces pombe* involved in DNA double-strand-break repair. Nucleic Acids Res. **20:** 6605–6611.

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

Boguski, M. S., and F. McCormick, 1993 Proteins regulating Ras and its relatives. Nature **366:** 643–654.

Corbett, A. H., D. M. Koepp, G. Schlenstedt, M. S. Lee, A. K. Hopper *et al.*, 1995 Rna1p, a Ran/TC4 GTPase activating protein, is required for nuclear import. J. Cell Biol. **130:** 1017–1026.

Dasso, M., 1995 The role of the Ran GTPase pathway in cell cycle control and interphase nuclear functions, pp. 163–172 in *Progress in Cell Cycle Research*, Vol. 1, edited by L. Meijer, S. Guidet and H. Y. L. Tung. Plenum Press, New York.

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

Gorlich, D., 1998 Transport into and out of the cell nucleus. EMBO J. **17:** 2721–2727.

Guacci, V., D. Koshland and A. Strunnikov, 1997 A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of *MCD1* in *S. cerevisiae*. Cell **91:** 47–57.

Gutz, H., H. Heslot, U. Leupold and N. Lopreno, 1974 *Schizosaccharomyces pombe*, pp. 395–446 in *Handbook of Genetics*, Vol. 1, edited by R. C. King. Plenum Press, New York.

Hartwell, L. H., 1967 Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93:** 1662–1670.

He, X., N. Hayashi, N. G. Walcott, Y. Azuma, T. E. Patterson *et al.*, 1998 The identification of cDNAs that affect the mitosis-to-interphase transition in *Schizosaccharomyces pombe*, including sbp1, which encodes a spi1p-GTP-binding protein. Genetics **148:** 645–656.

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

Kadowaki, T., S. Chen, M. Hitomi, E. Jacobs, C. Kumagai *et al.*, 1994 Isolation and characterization of *Saccharomyces cerevisiae* mRNA transport-defective (*mtr*) mutants. J. Cell Biol. **126:** 649–659.

Matsumoto, T., and D. Beach, 1991 Premature initiation of mitosis in yeast lacking RCC1 or an interacting GTPase. Cell **66:** 347–360.

Mattaj, I. W., and L. Englmeier, 1998 Nucleocytoplasmic transport: the soluble phase. Annu. Rev. Biochem. **67:** 265–306.

Matynia, A., K. Dimitrov, U. Mueller, X. He and S. Sazer, 1996 Perturbations in the spi1p GTPase cycle of *Schizosaccharomyces pombe* through its GTPase-activating protein and guanine nucleotide exchange factor components result in similar phenotypic consequences. Mol. Cell. Biol. **16:** 6352–6362.

Michaelis, C., R. Ciosk and K. Nasmyth, 1997 Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell **91:** 35–45.

Mitchell, P., E. Petfalski, A. Sehevchenko, M. Mann and D. Tollervey, 1997 The exosome: a conserved eukaryotic RNA processing complex containing multiple 3′->5′ exoribonucleases. Cell **91:** 457–466.

Moreno, S., A. Klar and P. Nurse, 1991 Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol. **194:** 795–823.

Noguchi, E., N. Hayashi, Y. Azuma, T. Seki, M. Nakamura *et al.*, 1996 Dis3, implicated in mitotic control, binds directly to Ran and enhances the GEF activity of RCC1. EMBO J. **15:** 5595–5605.

Oki, M., and T. Nishimoto, 1998 A protein required for nuclear-protein import, Mog1p, directly interacts with GTP-Gsp1p, the *Saccharomyces cerevisiae* Ran homologue. Proc. Natl. Acad. Sci. USA. **95:** 15388–15393.

Oki, M., E. Noguchi, N. Hayashi and T. Nishimoto, 1998 Nuclear protein import, but not mRNA export, is defective in all *Saccharomyces cerevisiae* mutants that produce temperature-sensitive forms of the Ran GTPase homologue Gsp1p. Mol. Gen. Genet. **257:** 624–634.

Ribbeck, K., G. Lipowsky, H. M. Kent, M. Stewart and D. Gorlich, 1998 NTF2 mediates nuclear import of Ran. EMBO J. **17:** 6587–6598.

Rothstein, R. J., 1983 One-step disruption in yeast. Methods Enzymol. **101:** 202–211.

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

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

Shibuya, T., S. Tsuneyoshi, A. K. Azad, S. Urushiyama, Y. Ohshima *et al.*, 1999 Characterization of the *ptr6*$^{+}$ gene in fission yeast: a possible involvement of a transcriptional coactivator TAF in nucleocytoplasmic transport of mRNA. Genetics **152:** 869–880.

Smith, A., A. Brownawell and I. G. Macara, 1998 Nuclear import of Ran is mediated by the transport factor NTF2. Curr. Biol. **8:** 1403–1406.

Steggerda, S. M., and B. M. Paschal, 2000 The mammalian Mog1 protein is a guanine nucleotide release factor for Ran. J. Biol. Chem. **275:** 23175–23180.

Stewart, M., and Baker, R. P., 2000 1.9 A resolution crystal structure of the *Saccharomyces cerevisiae* Ran-binding protein Mog1p. J. Mol. Biol. **299:** 213–223.

Tani, T., R. J. Derby, Y. Hiraoka and D. L. Spector, 1996 Nucleolar accumulation of poly (A)$^{+}$ RNA in heat-shocked yeast cells: implication of nucleolar involvement in mRNA transport. Mol. Biol. Cell **7:** 173–192.

Tatebayashi, K., J. Kato and H. Ikeda, 1998 Isolation of a *Schizosaccharomyces pombe rad21*^{ts} mutant that is aberrant in chromosome segregation, microtubule function, DNA repair and sensitive to hydroxyurea: possible involvement of Rad21 in ubiquitin-mediated proteolysis. Genetics **148:** 49–57.

van Hoof, A., P. Lennertz and R. Parker, 2000 Yeast exosome mutants accumulate 3′-extended polyadenylated forms of U4 small nuclear RNA and small nucleolar RNAs. Mol. Cell. Biol. **20:** 441–452.

Watanabe, Y., S. Shinozaki-Yabana, Y. Chikashige, Y. Hiraoka and M. Yamamoto, 1997 Phosphorylation of RNA-binding protein controls cell cycle switch from mitotic to meiotic in fission yeast. Nature **386:** 187–190.

Communicating editor: F. Winston