# Identification of novel suppressors for Mog1 implies its involvement in RNA metabolism, lipid metabolism and signal transduction

# Identification of novel suppressors for Mog1 implies its involvement in RNA metabolism, lipid metabolism and signal transduction

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**Abstract**

Mog1 is conserved from yeast to mammal, but its function is obscure. We isolated yeast genes that rescued a temperature-sensitive death of *S. cerevisiae Scmog1Δ*, and of *S. pombe Spmog1*$^{ts}$. *Scmog1Δ* was rescued by Opi3p, a phospholipid *N*-methyltransferase, in addition to *S. cerevisiae* Ran-homologue Gsp1p, and a RanGDP binding protein Ntf2p. On the other hand, *Spmog1*$^{ts}$ was rescued by Cid13 that is a poly (A) polymerase specific for *suc22*$^{+}$ mRNA encoding a subunit of ribonucleotide reductase, Ssp1 that is a protein kinase involved in stress response pathway, and Crp79 that is required for mRNA export, in addition to Spi1, *S. pombe* Ran-homologue, and Nxt2, *S. pombe* homologue of Ntf2p. Consistent with the identification of those suppressors, lack of ScMog1p dislocates Opi3p from the nuclear membrane and all of *Spmog1*$^{ts}$ showed the nuclear accumulation of mRNA. Furthermore, SpMog1 was co-precipitated with Nxt2 and Cid13.

*Keywords:* Cid13, Crp79, Ntf2p/Nxt2, Ran, Ssp1

## 1. Introduction

Ran GTPase mediates nucleoplasmic transport, microtubule assembly and nuclear membrane formation (Dasso, 2002; Weis, 2003; Mattaj, 2004). Guanine nucleotide triphophate (GTP) of Ran is hydrolized to guanine diphosphate (GDP) by the aid of RanGAP/Rna1, Ran GTPase activating protein. Ran-GDP is exchanged to Ran-GTP by RanGEF/RCC1, guanine nucleotide exchange factor. While RanGAP is mainly localized in the cytoplasm, RanGEF/RCC1 is localized in the nuclei, creating a gradient of Ran-GTP concentration from the nuclei to the cytoplasm that is important to determine the destination of nucleocytoplasmic transport. Proteins with a nuclear localization signal bind nuclear import receptors in the cytoplasm. Those complexes enter nucleus through the nuclear pore complexes (NPC) (Weis, 2003). In the nucleus, Ran-GTP binds nuclear import receptors, releasing cargo-proteins from import-complexes. On the other hand, proteins with a nuclear export-signal are transported to the cytoplasm by the export receptors that bind Ran-GTP. In the cytoplasm, GTP of exported Ran-GTP is hydrolysed by the aide of RanGAP, terminating the nuclear export. Resulting Ran-GDP is imported to the nucleus by *NTF2*, nuclear transport factor No. 2 (Ribbeck et al., 1998; Smith et al., 1998). In the nucleus, GDP of Ran-GDP is exchanged

*Abbreviations:* ts, temperature-sensitive; Mog1, multicopy suppressor of ts gsp1; Opi3, overproducer of inositol; NTF2, nuclear transport factor; GST, glutathione *S*-transferase; DAPI, 4'6-diamidino-2-phenylindole.

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Table 1
Suppressor genes for *Scmog1Δ* and *Spmog1*$^{ts}$

| Suppressor genes | Frequency* |
|---|---|
| For *Scmog1Δ* | |
| *MOG1* | 65 |
| *GSP1* | 7 |
| *NTF2* | 2 |
| *GSP2* | 6 |
| *OPI3* | 19 |
| *For Spmog1*$^{ts}$ | |
| *mog1*$^{+}$ | 19 |
| *spi1*$^{+}$ | 56 |
| *nxt2*$^{+}$ | 17 |
| *cid13*$^{+}$ | 2.0 |
| *ssp1*$^{+}$ | 1.6 |
| *crp79*$^{+}$ | 1.0 |
| *SPBC354.10*$^{+}$ | 3.2 |

*Ratio (%) for the total number of independently isolated suppressor genes.

with GTP, releasing *NTF2* that will be backed to the cytoplasm (Stewart, 2000). *NTF2*, an essential protein for nuclear protein import (Moore and Blobel, 1994; Paschal and Gerace, 1995), is thought to function solely for nuclear import of Ran-GDP. In this regard, *NTF2* that specifically binds Ran-GDP is argued to be a Ran-GDP dissociation inhibitor (GDI) (Yamada et al., 2004).

Mog1 was first identified as a suppressor that rescues a temperature-sensitive defect of *S. cerevisiae* Ran, Gsp1p, *gsp1*$^{ts}$ that can be also rescued by *S. cerevisiae NTF2*, Ntf2p (Oki and Nishimoto, 1998). Later on, Mog1 is found to be well conserved from yeast to human. Both ScMog1p and mammalian Mog1 bind Ran-GTP. They showed the activity of GTP releasing, but not, that of nucletide exchanging of RanGTP (Steggerda and Paschal, 2000; Oki and Nishimoto, 2000). In this regard, Mog1 is suggested to enhance the GDP→GTP exchange by collaborating with RanGEF/RCC1 and RanBP1 (Nicolas et al., 2001), accumulating Ran-GTP in the nucleus to accelerate the nucleocytoplasmic transport. Consistently, when overexpressed, ScMog1p is localized in the nucleus of both yeast and mammals (Oki and Nishimoto, 1998; Tatebayashi et al., 2001; Marfatia et al., 2001) and lack of Mog1p causes nuclear import defect (Oki and Nishimoto, 1998). In *S. pombe,* however, a nuclear import defect is suggested due to a loss of nuclear envelope integrity that happens in *Spmog1-1*$^{ts}$ (Tatebayashi et al., 2001). On the other hand, ScMog1p is reported to be involved in *S. cerevisiae SLN1-SKN7* signal transduction (Lu et al., 2004), indicating that Mog1p may function not only in the nucleus, but also in the cytoplasm. Unexpectedly, the crystal structure of ScMog1p is reported to be similar to PsbP, a membrane-extrinsic subunit of photosystem II from *N. tabacum*, the hypothetical protein Pa94 from *P. aeruginosa* and TM1622, a hypothetical protein from Thermotoga maritime (Ifuku et al., 2004; Xu et al., 2006). These curious reports force to clarify a biological function of Mog1. Presently we isolated suppressors that rescued a defect of Mog1 using both *S. cerevisiae* and *S. pombe*. In both cases, Ntf2p and *S. pombe NTF2*-homologue, Ntx2 were obtained as a suppressor. While a major suppressor for *Spmog1*$^{ts}$ was Ntx2, the phospholipids *N*-methyltransferase Opi3p mainly suppressed *Sc mog1Δ*.

## 2. Materials and methods

### 2.1. Yeast media and strains

*S. cerevisiae Scmog1Δ* (MOY1:*MATα mog1Δ::HIS3, ade2-101, leu2-Δ1, trp1-Δ63, ura3-52, lys2-801, his3-Δ200*) (Oki and Nishimoto, 1998) was used to isolate suppressors for loss of

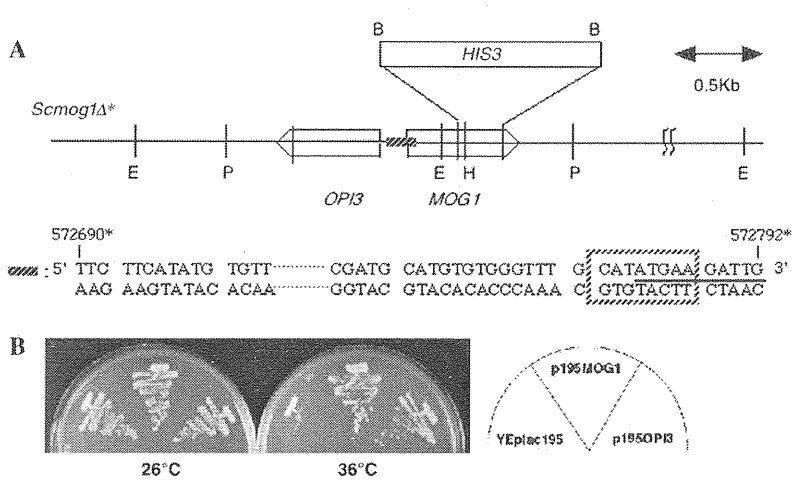


Fig. 1. *OPI3* rescued a temperature-sensitive lethality of *Scmog1Δ*$^{*}$. A. Construction of *Scmog1Δ*$^{*}$. The plasmid, pHIS3MOG1N (Oki and Nishimoto, 1998) digested with *Xba*I and *Xho*I was introduced into YPH499 (Oki and Nishimoto, 1998), resulting in *Scmog1Δ*$^{*}$. Nucleotide sequence of the oblique striped region was shown: the squared UAS of *OPI3* overlaps with the ORF of Mog1p (underlined). *: The nucleotide number on *S. cerevisiae* chromosome X. B. The indicated plasmids, YEplac195 (*2μ URA3*), p195MOG1 (YEplac195 containing *MOG1*) (Oki and Nishimoto, 1998) and p195OPI3 (YEplac195 containing *OPI3*) were introduced into *Scmog1Δ*$^{*}$. URA$^{+}$ transformants were incubated on a synthetic medium lacking uracil at the indicated temperature.

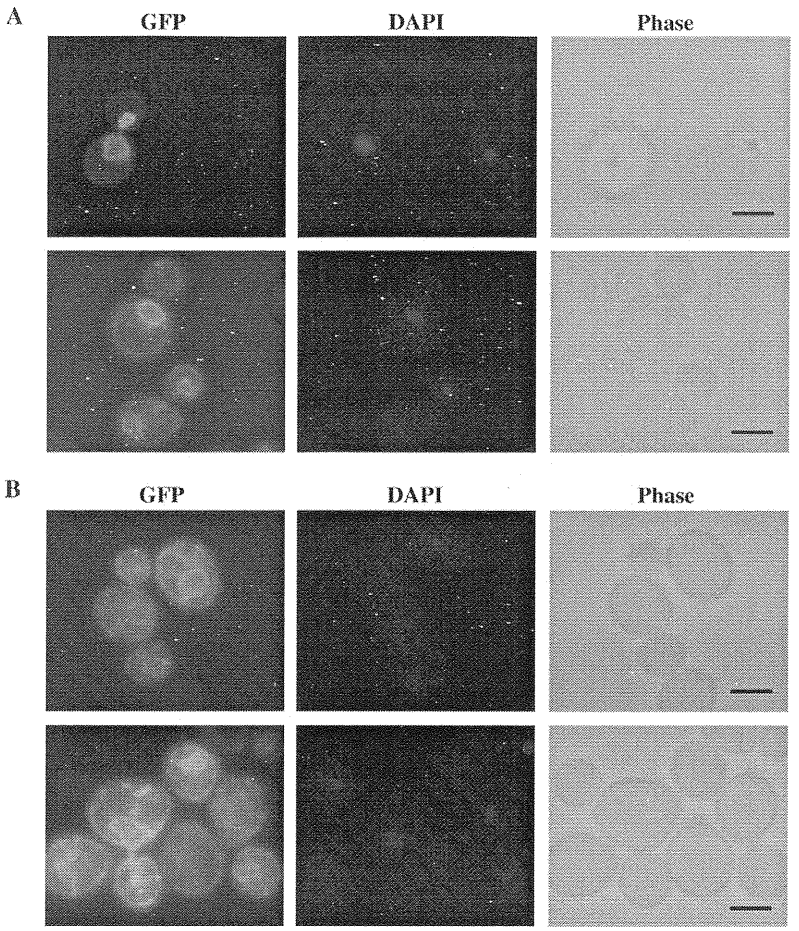


Fig. 2. Localization of OPI3p. Exponentially growing *S. cerevisiae* MOY11 [p314EGFP-OPI3] (A) and *S. cerevisiae* MOY12 [p314EGFP-OPI3] (B) were fixed with paraformaldehyde. GFP was observed by fluorescence microscopy. DNA was stained with DAPI. Representative figures are shown. Bar: 2 μm.

*Sc*Mog1p. *S. pombe* diploid strain (*h*$^{+}$/*h*$^{-}$ *ade6-M216/ade6-M210, leu1-32/leu1-32, ura4-Δ18/ura4- Δ18, his7-366/his7-366, mog1*$^{+}$*/mog1::ura4*$^{+}$) (Tatebayashi et al., 2001) was used to isolate *S. pombe mog1*$^{ts}$ that was then used for suppressor-isolation. *S. cerevisiae* and *S. pombe* strains were grown in medium with appropriate supplements as described by Oki and Nishimoto (1998), and Kusano et al. (2004), respectively. The following new *S. cerevisiae* strains were constructed for this study: MOY11 (*MAT*a *opi3Δ::HIS3, ade2–101, leu2-Δ1, trp1-Δ63, ura3–52, lys2–801, his3-Δ200*), MOY12 (*MAT*a *opi3Δ::HIS3, mog1Δ::HIS3, ade2-101, leu2-Δ1, trp1-Δ63, ura3-52, lys2-801, his3-Δ200*).

### 2.2. Isolation of *Spmog1*$^{ts}$

*Spmog1*$^{+}$ genomic DNA was amplified by PCR using following primers: 5′ primer, AAA CTC GAG ATG GTA CAG CTA TTC GGT GGG GCT and 3′ primer, CGC GGA TCC GCC TAT GTC CAT CAT CCT TTA AGC. Amplified DNA fragments of 0.6 kilobase pair (kb) were inserted into a vector, pREP81X (Maundrell, 1993). Resulting plasmid, pREP81X-Spmog1 was introduced into *S. pombe* diploid strain (*mog1*$^{+}$/*mog1::ura4*$^{+}$) to isolate *S. pombe* haploid strain, *Spmog1::ura4*$^{+}$ [pREP81X-Spmog1]. To introduce the mutation, the *Spmog1*$^{+}$ genomic DNA fragment was amplified in the presence of an appropriate concentration of $Mn^{2+}$, using primer sets: 5′ primer, GGT TGA ATA CTT ATT ATC TAG TGA ACG and 3′ primer, TCT TCT TCG TAT GAT GAT ATC CT, as described (Hirose et al., 2006), and then transfered into *Spmog1::ura4*$^{+}$ [pREP81X-Spmog1] through electroporation. Transfected cells papillated on plates containing adenine,

Table 2
*SpMog1*$^{ts}$ and its mutation site

| *Spmog1*$^{ts}$[1] | Amino acid change[2] |
|---|---|
| *Spmog1-2*$^{ts}$ | S180P |
| *Spmog1-3*$^{ts}$ | W79R |
| *Spmog1-4*$^{ts}$ | L9H |
| *Spmog1-5*$^{ts}$ | F5L |
| *Spmog1-6*$^{ts}$ | F5S |

[1] *Spmog1-1*$^{ts}$ was reported by Tatebayashi et al., 2001.
[2] Amino acid was shown by a single letter.

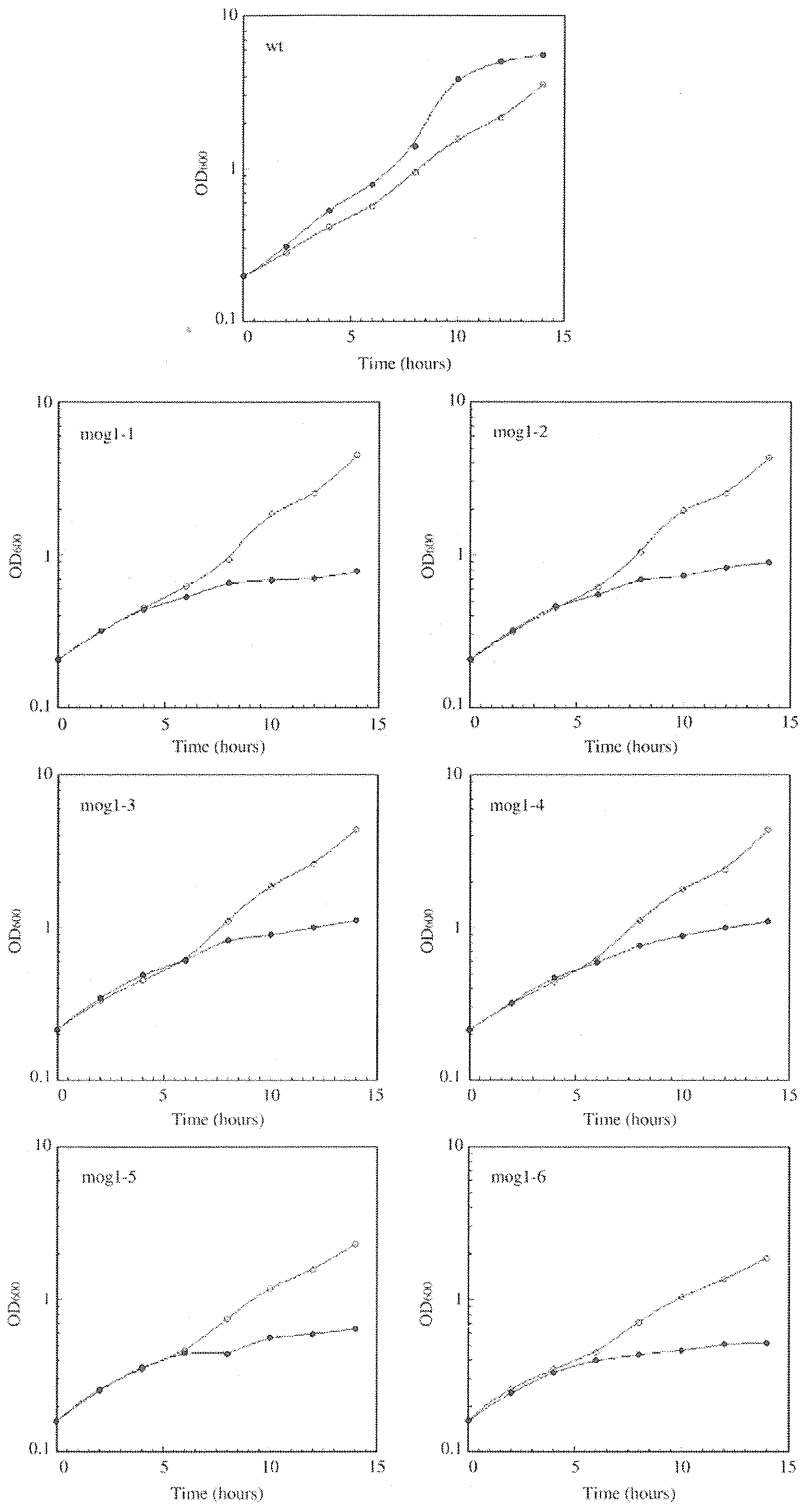


Fig. 3. Growth curve of *Spmog1*$^{ts}$. Overnight cultures of *Spmog1*$^{wt}$ and *Spmog1*$^{ts}$ strains were diluted into YES medium and grown at 26 °C until $OD_{600\ nm}$=0.2. Half of each culture was then shifted to 37 °C (filled circles) and the other half was further incubated at 26 °C (open circle).

histidine, uracil, leucine, thiamine and 5-fluoroorotic acid (5-FOA) at 26 °C, were replica-plated on YE5S phloxinB plates, and then incubated at 26 °C or 36 °C. Temperature sensitive strains were backcrossed with *Spmog1*$^{+}$.

### *2.3. Isolation of multicopy suppressors*

For *Scmog1Δ*: the genomic library (RB236) constructed by using the vector YEp24 (Carlson and Botstein, 1982), was

introduced into cultures of *Scmog1Δ*. Plasmids were isolated from *ts*$^{+}$ *URA*$^{+}$ transformants using *E. coli* as described (Oki and Nishimoto, 1998). *S. cerevisiae* genomic DNA of recovered plasmid DNA was sequenced to search for an open reading frame (ORF) using the MIPS program (Munich Information Center for Protein Sequence). The *ORF*s carried on *S. cerevisiae* genomic DNA fragments were inserted into YEplac195 to introduce into *Scmog1Δ*.

For *Spmog1*$^{ts}$: *S. pombe* genomic library constructed by using the vector pAL-SK, was kindly provided from Dr. M. Yanagida (Kyoto University) and introduced into both *Spmog1-3*$^{ts}$ and *Spmog1-4*$^{ts}$ (Table 1) with electroporation. *S. pombe genomic* DNA recovered from *ts*$^{+}$ transformants as described (Kusano et al., 2004), was sequenced. All ORFs found in recovered *S. pombe genomic* DNA fragments were subcloned into the vector pAL-SK and then introduced into *Spmog1*$^{ts}$.

### *2.4. mRNA export assay*

The mRNA export assay was performed as described (Tani et al., 1996).

## 3. Results

### *3.1. OPI3 rescued Scmog1Δ*

As reported (Oki and Nishimoto, 1998), *S. cerevisiae* Ran, Gsp1p and Gsp2p (Belhumer et al., 1993), and Ntf2p (Paschal and Gerace, 1995), rescued a temperature sensitive death of *Scmog1Δ* lacks *ScMOG1*, indicating the genetic system to isolate suppressor genes of *MOG1* is working for *Scmog1Δ*. Opi3p that is the phospholipids *N*-methyltransferase (Greenberg et al., 1982; Boumann et al., 2004) was obtained as a major suppressor for *Scmog1Δ* (Table 1). *S. cerevisiae* genomic DNA sequence revealed the upstream activation sequence (UAS) of *OPI3* (Kodaki et al., 1991) overlaps with *ScMOG1*-ORF as shown in Fig. 1A, so that it was deleted in *Scmog1Δ*. Since UAS is essential for an efficient gene expression, we constructed a new null mutant of *ScMOG1*, *Scmog1Δ** (MOY7: *MAT*a *mog1Δ::HIS3, ade2-101, leu2-Δ1, trp1-Δ63, ura3-52, lys2-801, his3-Δ200*), that lacks the C-terminal region of *ScMOG1*, but possess the UAS sequence of *OPI3* (Fig. 1A). Opi3p also rescued a temperature-sensitive death of *Scmog1Δ** (Fig. 1B), indicating it is a novel suppressor of *Scmog1Δ*. When overexpressed, Opi3p was localized on the periphery of *S. serevisiae* nucleus (Fig. 2A). Interestingly, Opi3p was dislocated from the nuclear membrane in the absence of ScMog1p (Fig. 2B).

### *3.2. Suppressors for Spmog1*$^{ts}$

Proteins directly interacting with Mog1p may not be obtained as a suppressor of *Scmog1Δ* that lacks ScMog1p. In order to overcome this issue, we took the advantage of *S. pombe* that *Spmog1*$^{+}$ is essential for cell-survival (Tatebayashi et al., 2001). A series of temperature-sensitive mutants of *Spmog1*$^{+}$ were isolated using the error prone PCR as described (Oki et al., 1998). Among obtained mutants, five *Spmog1*$^{ts}$ strains that possess a single amino acid change, were chosen for a further analysis (Table 2). Their growth curves were shown in Fig. 3. *S. pombe* genomic libraries were introduced into *Spmog1-3*$^{ts}$ and *Spmog1-4*$^{ts}$. Both *spi1*$^{+}$ and *nxt2*$^{+}$ were isolated as suppressor genes of *Spmog1*$^{ts}$ with high frequencies (Table 1). Spi1 is *S. pombe* Ran homologue (Matsumoto and Beach, 1991) and Nxt2, *NTF2*-related export protein 2, is 69% identical to Ntf2p (Black et al., 1999; BLAST Alignment for *S. cerevisiae NTF2* vs *S. pombe HITS*). Additionally, a temperature-sensitive growth of *Spmog1*$^{ts}$ was rescued by Cid13, poly (A) polymerase for *suc22*$^{+}$ mRNA (Saitoh et al., 2002); Crp79, mRNA export carrier (Thakurta et al., 2002); Ssp1, a protein kinase involved in alteration of cell polarity (Matsusaka et al., 1995; Rupes et al., 1999) and the ORF: SPBC354.10 that is a hypothetical protein (Table 1). A representative result of suppression was shown in Fig. 4. There was no *cho1*$^{+}$, encoding a *S. pombe* homologue of *S. cerevisiae* Opi3p (Kanipes et al., 1998a,b) (Table 1).

### *3.3. mRNA export defect of Spmog1*$^{ts}$

Both Cid13 and Crp79 suggest an involvement of Mog1 in mRNA metabolism. Previous reports, however, are controversial.

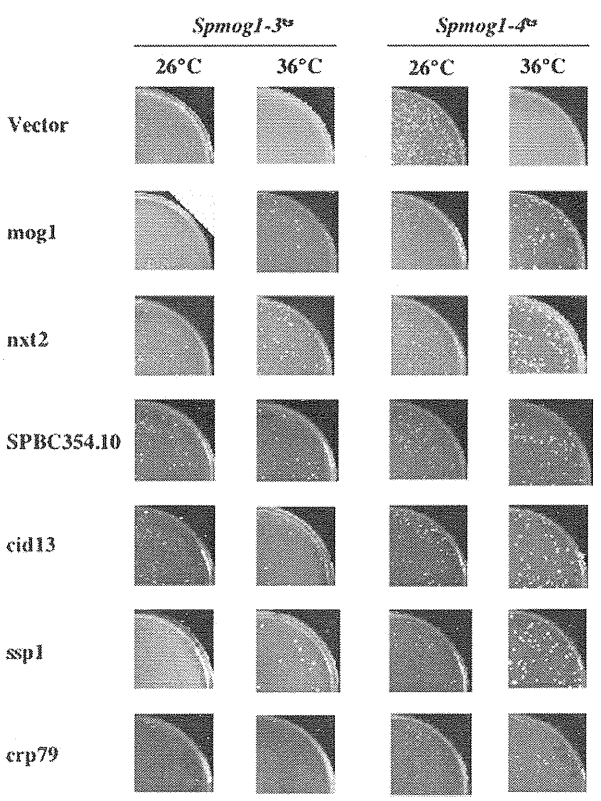


Fig. 4. A temperature sensitive lethality of *Spmog1*$^{ts}$ was rescued by isolated suppressors. The vector, pAL-SK, and the indicated suppressor genes containing their own promoters carried on pAL-SK were introduced into *Spmog1-3*$^{ts}$ and *Spmog1-4*$^{ts}$, respectively. Resulting transformants were incubated on YE5S plates supplemented with His, Ade and Ura at the indicated temperature.

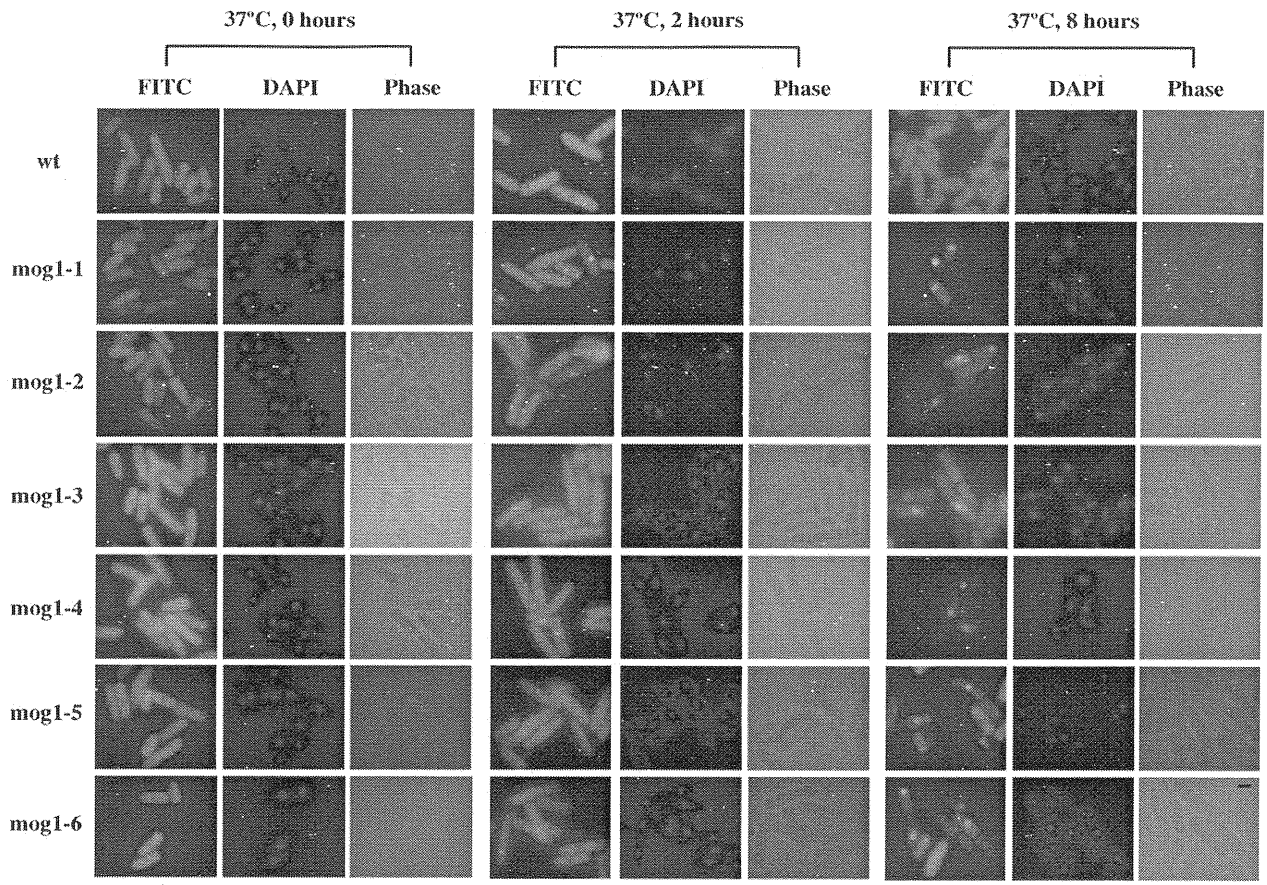


Fig. 5. mRNA export assay. *Spmog1*$^{wt}$ and *Spmog1*$^{ts}$ were cultured in YES medium at 26 °C until $OD_{600\ nm}$=0.2, and the half of cultures were incubated at 37 °C (see Fig. 3). Cells were fixed at the indicated time and hybridized with biotin-labeled oligo$(dT)_{49}$, followed by incubation with FITC-avidin. Cellular DNA was stained with DAPI. FITC and DAPI of the same fields of cells are shown, together with the corresponding phase contrast views. Bar: 2 μm.

*Scmog1Δ* shows a normal mRNA export (Oki and Nishimoto, 1998), but *Spmog1-1*$^{ts}$ has a defect in mRNA export (Tatebayashi et al., 2001). To clarify this issue, mRNA export of all five *Spmog1*$^{ts}$ mutants was examined as shown in Fig. 5. The frequency of cells showing the nuclear accumulation of poly (A) RNA was calculated in Table 3. These data indicated that all of *Spmog1*$^{ts}$ mutants clearly showed mRNA export defect after 2 h incubation at 37 °C when they were still growing (Fig. 3).

### *3.4. Nxt2 and Cid13 were co-immunoprecipitated with SpMog1*

The next question is whether suppressors of *Spmog1*$^{ts}$ directly interact with SpMog1 or not. To address this issue, obtained suppressor genes and *Spmog1*$^{+}$ were tagged with Myc and HA (hemagglutinin), respectively. Myc-tagged suppressor genes and HA-tagged *Spmog1*$^{+}$ were simultaneously introduced into *Spmog1*$^{wt}$. After expression of the introduced genes in the absence of thiamine, HA-SpMog1 was pulled down using the mAb to HA. Proteins that were co-immunoprecipitated with HA-SpMog1, were analyzed by immunoblotting with the mAb to Myc. Myc-tagged Spi1 and anti-GST antibody were used as a positive and negative control. As reported for *S. cerevisiae* Ran Gsp1p (Oki and Nishimoto, 1998), *S. pombe* Ran Spi1 was co-immunoprecipitated with SpMog1. Under the same condition, both Nxt2 and Cid13 were apparently co-immunoprecipitated with SpMog1 (lane 3),

Table 3
The frequency of cells (%) showing the nuclear accumulation of poly (A) RNA

| | Time[1] | | |
|---|---|---|---|
| *Spmog1*$^{ts}$ | 0 h | 2 h | 8 h |
| *wt* | 0.0 | 0.0 | 0.6 |
| *Spmog1-1*$^{ts}$ | 2.4 | 92.1 | 64.4 |
| *Spmog1-2*$^{ts}$ | 0.6 | 79.2 | 73.6 |
| *Spmog1-3*$^{ts}$ | 0.0 | 24.2 | 76.0 |
| *Spmog1-4*$^{ts}$ | 1.9 | 70.3 | 74.1 |
| *Spmog1-5*$^{ts}$ | 4.6 | 58.2 | 65.5 |
| *Spmog1-6*$^{ts}$ | 3.4 | 69.9 | 37.1 |

[1]After shift up to 37 °C.
%: more than 150 cells were examined for the nuclear accumulation of poly (A) RNA.

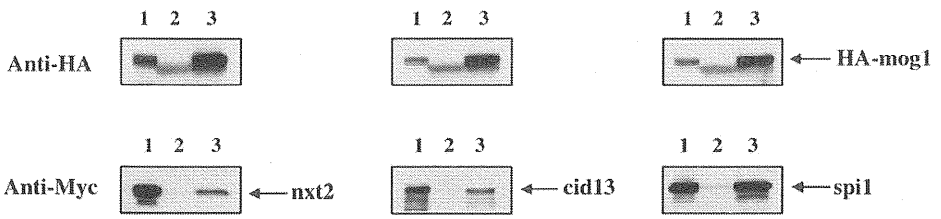


Fig. 6. Mog1 interacted with Nxt2, Cid13 and Spi1. HA-Mog1 carried on the vector, pRHA42, and the suppressors, Myc-Nxt2, Myc-Cid13 and Myc-Spi1 carried on the vector, pRMH41, were co-expressed in *Spmog1*$^{+}$ (*h*$^{+}$ *ade6 m-216 leu1-32 ura4-Δ18*) (Kusano et al., 2004) in medium lacking thiamine. Exponentially growing cells were collected and cell-lysates were prepared as described (Nishijima et al., 2006). From resulting cell-lysates, HA-Mog1 was pulled down using anti-HA antibody (clone HA-7). Proteins co-immunoprecipitated with HA-Mog1 were separated with SDS-PAGE and analyzed by immunoblotting with the mAb to Myc (clone 9E10). 1. Total lysates; 2. Pulled down with anti-GST-antibody as a control; 3. Pulled down with the mAb to HA.

compared with the total lysates (lane 1) and the fraction precipitated by the anti-GST antibody (lane 2)(see Fig. 6).

## 4. Discussion

Opi3p is a phospholipid methyltransferase required for the synthesis of phosphatidylcholine that is a major phospholipid constituent of eukaryotic membrane (Boumann et al., 2004). The finding that overexpression of Opi3p rescued *Scmog1Δ*, thus, suggests that Mog1p is involved in membrane formation. Consistently, lack of *MOG1* seems to cause membrane fragile since the cellular body of *Scmog1Δ* was enlarged (Oki and Nishimoto, 1998) and the nuclear envelope of *Spmog1-1*$^{ts}$ was reported to be abnormal (Tatebayashi et al., 2001). Opi3p that is localized in the nuclear periphery, might contribute to strength cellular membrane through the synthesis of phosphatidylcholine. How *MOG1* is involved in membrane formation is an intriguing future question. Our present result indicated that ScMog1p is required for a membrane localization of Opi3p.

Nxt2 is most homologous to Ntf2p among *S. pombe* Nxt proteins; Nxt1, Nxt2 and Nxt3, all of which possess the Ntf2p domain (Black et al., 1999; BLAST Alignment for *S. cerevisiae NTF2* vs *S. pombe HITS*). The fact that only Nxt2 out of the Nxt family was obtained as a suppressor of *Spmog1*$^{ts}$ even with the same frequency as SpMog1, reveals a tight functional relationship of Mog1p with Ntf2p. Indeed, SpMog1 was co-precipitated with Nxt2. Mog1p is structurally similar to Ntf2p that make a dimer to transport RanGDP into the nucleus (Stewart and Baker, 2000; Chaillan-Huntington et al., 2001). Since the dimer formation of Ntf2p depends on the Ntf domain, Ntf2p is suggested to make a heterodimer with Tap1 and Nxt1 that contains a domain homologus to Ntf2p (Chaillan-Huntington et al., 2001). In this regard, Mog1p could make a heterodimer with Ntf2p as suggested by the fact that SpMog1 was co-immunoprecipitated with Nxt2. Since *NTF2* rescued *Scmog1Δ*, a heterodimer of *NTF2* and *MOG1* might be substituted by a homodimer of *NTF2*. Whether SpMog1 could be co-precipitated with Nxt1 or Nxt3 remains to be investigated.

The other suppressors that were obtained with a low frequency, apparently rescued *Spmog1*$^{ts}$. Furthermore, Cid13 was co-immunoprecipitated with SpMog1, revealing a tight interaction of SpMog1 with Cid13. Since Cid13 is a poly (A) polymerase for *suc22* mRNA that encodes a subunit of ribonucleotide reductase, Cid13 is assumed to regulate the S–M transition (Saitoh et al., 2002). Taken together these reports regarding Cid13 with the previous report that *Spmog1*$^{ts}$ is arrested with G2-M DNA content (Tatebayashi et al., 2001), Mog1 may regulate the cell cycle progression through Cid13. To prove this issue, we tried to determine whether the amount of *suc22* mRNA was reduced in *Spmog1*$^{ts}$. So far, we could not get a clear answer yet. Thus the question of how SpMog1 functionally interacts with Cid13, remains to be investigated. Crp79 functions for mRNA export like Mex67 that interacts with p15/Nxt3 (Thakurta et al., 2002). Thus, it is possible that Crp79 interacts with Mog1 through Nxt3. The fact that both Cid13 and Crp79 were isolated as a suppressor of *Spmog1*$^{ts}$, supports the idea that Mog1 is involved in mRNA metabolism, as reported previously (Tatebayashi et al., 2001). Indeed, we confirmed all of *Spmog1*$^{ts}$ mutants have a defect in mRNA export.

Ssp1 is a protein kinase involved in stress response pathway (Matsusaka et al., 1995; Rupes et al., 1999). The fact that Ssp1 suppressed *Spmog1*$^{ts}$, therefore, is consistent with the previous report that SpMog1 is involved in stress response pathway (Lu et al., 2004). It remains to be examined whether Crp79, Ssp1 and the protein encoded by ORF: SPBC354.10 could be co-immunoprecipitated with SpMog1.

In conclusion, our results indicate Mog1 functions not only for nuclear import but also for RNA metabolism and signal transduction.

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## References

Belhumer, P., et al., 1993. GSP1 and GSP2, genetic suppressors of the prp20-1 mutant in Saccharomyces cerevisiae; GTP-binding proteins involved in the maintenance of nuclear organization. Mol. Cell Biol. 13, 2152–2161.

Black, B., Levesque, L., Holaska, J.M., Wood, T.C., Paschal, B.M., 1999. Identification of an NTF2-related factor that binds Ran-GTP and regulates nuclear protein export. Mol. Cell. Biol. 19, 8516–8621.

Boumann, H.A., Chin, P.T.K., Heck, A.J.R., de Kruijff, B., de Kroon, A.I.P.M., 2004. The yeast phospholipids *N*-methyltransferases catalyzing the synthesis of phospatidylcholine preferentially convert Di-Cl 6:1 substrates both in vivo and in vitro. J. Biol. Chem. 279, 40314–40319.

Carlson, M., Botstein, D., 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted with intracellular forms of yeast invertase. Cell 28, 145–154.

Chaillan-Huntington, C., et al., 2001. NTF2 monomer-dimer equilibrium. J. Mol. Biol. 314, 465–477.

Dasso, M., 2002. The Ran GTPase: theme and variations. Curr. Biol. 12, R502–R508.

Greenberg, M.L., Reiner, B., Henry, S.A., 1982. Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol-excreting mutants. Genetics 100, 19–33.

Hirose, E., et al., 2006. Loss of RanGEF/Pim1 activity abolishes the orchestration of Ran-mediated mitotic cellular events in *S pombe*. Genes Cells 11, 29–46.

Ifuku, K., Nakatsu, T., Kato, H., Sato, F., 2004. Crystal structure of the PasbP protein of photosystem II from *Nicotiana tabacum*. EMBO Rep. 5, 362–367.

Kanipes, M.I., Hill, J.E., Henry, S.A., 1998a. The *Schizosaccharomyces pombe* cho1+ gene encodes a phospholipids methytransferase. Genetics 150, 553–562.

Kanipes, M.I., Hill, J.E., Henry, S.A., 1998b. The *Schizosaccharomyces pombe* cho1+ gene encodes a phospholipid methyltransferase. Genetics 150, 553–562.

Kodaki, T., Hosaka, K., Nikawa, J., Yamashita, S., 1991. Identification of the upstream activation sequences responsible for the expression of the phosphatidylethanolamine methylation pathway in *Saccharomyces cerevisiae*. J. Biochem. 109, 276–287.

Kusano, A., Yoshioka, T., Nishijima, H., Nishitani, H., Nishimoto, T., 2004. Schizosaccharomyces pombe RanGAP homolog, SpRna1, is required for centromeric silencing and chromosome segregation. Mol. Biol. Cell 15, 4960–4970.

Lu, J., M-Y., Deschenes, R.J., Fassler, J.S., 2004. Role for the Ran Binding Protein, Mog1p, in *Saccharomyces cerevisiae* SLN1-SKN7 Signal transduction. Eukaryotic Cell 3, 1544–1556.

Marfatia, K.A., Harreman, M.T., Fanara, P., Vertino, P.M., Corbett, A.H., 2001. Identification and characterization of the human MOG1 gene. Gene 266, 45–56.

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

Matsusaka, T., Hirata, D., Yanagida, M., Toda, T., 1995. A novel protein kinase gene ssp1+ is required for alteration of growth polarity and actin localization in fission yeast. EMBO J. 14, 3325–3338.

Mattaj, I., 2004. Sorting out the nuclear envelope from the endoplasmic reticulum. Nat. Rev., Mol. Cell Biol. 5, 65–69.

Maundrell, K., 1993. Thiamine-repressible expression vectors pREP and pRIP for fission yeast. Gene 123, 127–130.

Moore, M.S., Blobel, G., 1994. Purification of Ran-interacting protein that is required for protein import into the nucleus. Proc. Natl. Acad. Sci. U. S. A. 91, 10212–10216.

Nishijima, H., et al., 2006. Nuclear RanGAP is required for the heterochromatin assembly and is reciprocally regulated by histone H3 and Clr4 histone methyltransferase in *Schizosaccharomyces pombe*. Mol. Biol. Cell 17, 2524–2536.

Nicolas, F.J., Moore, W.J., Zhang, C., Clarke, P.R., 2001. Xmog1, a nuclear Ran-binding protein in Xenopus, is a functional homologue of *Schizosaccharomyces pombe* Mog1p that co-operates with RanBP1 to control generation of Ran-RanGTP. J. Cell Sci. 114, 3013–3023.

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

Oki, M., Nishimoto, T., 2000. Yrb1p interaction with the Gsp1p C terminus blocks Mog1p stimulation of GTP release from Gsp1p. J. Biol. Chem. 275, 32894–32900.

Oki, M., Noguchi, E., Hayashi, N., Nishimoto, T., 1998. Nuclear protein import, but not mRNA export, is defective in all of the temperature-sensitive mutants of the *Saccharomyces cerevisiae* Ran homologue, Gsp1-GTPase. Mol. Gen. Genet. 257, 624–634.

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

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

Rupes, I., Jia, Z., Young, P.G., 1999. Ssp1 promotes action depolymerization and is involved in stress response and new end take-off control in fission yeast. Mol. Biol. Cell 10, 1495–1510.

Saitoh, S., et al., 2002. Cid13 is a cytoplasmic poly (A) polymerase that regulates ribonucleotide reductase mRNA. Cell 109, 563–573.

Smith, A., Brownawall, A., Macara, I.G., 1998. Nuclear Import of Ran is mediated by the transport factor NTF2. Curr. Biol. 8, 1376–1386.

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

Stewart, M., 2000. Mechanism of Nuclear Trafficking using Nuclear Transport Factor 2 (NTF2). Cell Struct. Funct. 25, 217–225.

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

Tani, T., Derby, R.J., Hiraoka, Y., Spector, D.L., 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., Tani, T., Ikeda, H., 2001. Fission yeast Mog1p homologue, which interacts with the small GTPase Ran, is required for mitosis-to-interphase transition and poly(A) RNA metabolism. Genetics 157, 1512–1522.

Thakurta, A.G., et al., 2002. Crp79p, like Mex67p, is an auxiliary mRNA export factor in *Schizosaccharomyces pombe*. Mol. Biol. Cell 13, 2571–2584.

Xu, Q., et al., 2006. Crystal structure of an ORFan protein (TM1622) from thermotoga maritime at 1.75 A resolution reveals a fold similar to the Ran-binding protein Mog1p. PROTEINS 65, 777–782.

Weis, K., 2003. Regulating access to the genome: nucleocytoplasmic transport throughout the cell cycle. Cell 112, 441–451.

Yamada, M., Mattaj, I., Yoneda, Y., 2004. An ATP-dependent Activity that release RanGDP from NTF2. J. Biol. Chem. 279, 36228–36234.