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Isolation of a Novel *rmn1* Gene Genetically Linked to *spnab2* with Respect to mRNA Export in Fission Yeast

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In fission yeast, *Schizosaccharomyces pombe*, the *spnab2* gene encodes an ortholog of the budding yeast nuclear abundant poly(A)$^+$ RNA-binding protein 2 (Nab2) that is an essential protein required for both mRNA biogenesis and nuclear export of mRNA to the cytoplasm. We have previously isolated three mutants (SLnab1-3) that showed synthetic lethality under the repressed condition of *spnab2* expression. In this study, we isolated a novel *rmn1* gene as a multicopy suppressor that complemented the defects in growth and mRNA export of SLnab1 mutant cells. The *rmn1* gene contained three introns and encoded a 589 amino-acid protein with the RNA recognition motif (RRM) in the central region. The *Δrmn1* null mutant was viable but showed a slight mRNA export defect. However, its overexpression caused a deleterious effect on growth accompanied by intense accumulation of poly(A)$^+$ RNA in the nucleus. The combination of *Δrmn1* with *Δspnab2* or *Δspmex67* also inhibited growth. In addition, Rmn1p was associated with Rae1p *in vivo*. These results suggest that *rmn1* is a novel gene that is functionally linked to *spnab2*.

INTRODUCTION

For adequate gene expression in eukaryotes, pre-mRNAs in eukaryotes must be properly processed within the nucleus before export to the cytoplasm for translation into proteins. As soon as they emerge from RNA polymerase II, nascent mRNA transcripts successively undergo a series of co-transcriptional processing steps (5′-end capping, splicing, and 3′-end cleavage followed by polyadenylation) and are packaged into messenger ribonucleoprotein (mRNP) complexes in the nucleus (Kelly and Corbett, 2009). The export-competent mRNP complexes, containing the fully processed mature mRNA, are then translocated through the nuclear pore complex (NPC) and the export complexes are disassembled in the cytoplasm (Fasken and Corbett, 2009; Stewart, 2010). The export of bulk mRNA through NPCs is thought to be mediated primarily by the evolutionarily conserved Mex67-Mtr2 heterodimer in budding yeast, *Saccharomyces cerevisiae*, which promotes mRNA export through interactions with both mRNPs and nuclear pore proteins (Segref et al., 1997; Stewart 2010; Terry and Wente, 2007). In addition, defective mRNAs that are improperly processed or packaged are retained in the nucleus and eventually degraded by the surveillance mechanism for mRNA quality control (Schmid and Jensen, 2010). The nuclear steps from transcription to mRNA export are closely integrated, and failure of any one of these steps could affect the others. These mRNA biogenesis and export steps are controlled by RNA binding proteins, which are recruited to maturing mRNAs during transcription or mRNA processing (Fasken and Corbett, 2009; Stewart 2010; Tutucci et al., 2011).

The *S. cerevisiae* Nab2 is one of several factors that contribute to the coupling of mRNA biogenesis and export steps. Nab2 was first identified as a poly(A)$^+$ RNA-binding protein (Anderson et al., 1993), which binds specifically to polyadenosine RNA via a CCCH-type zinc finger domain *in vivo* and *in vitro* (Hector et al., 2002; Marfatia et al., 2003; Viphakone et al., 2008). Nab2 is essential for viability and is important for regulating the length of the mRNA poly(A) tail, protecting their 3′-ends, and impeding excessive polyadenylation mediated by Pab1 (Hector et al., 2002; Soucek et al., 2012; Viphakone et al., 2008). Additionally, Nab2 is an essential mRNA export protein that shuttles between the nucleus and the cytoplasm (Duncan et al., 2000; Green et al., 2002) and interacts functionally and physically with other mRNA export factors at multiple steps of mRNA export. Accordingly, Nab2 seems to function as a adaptor that recruits the mRNA export receptor Mex67-Mtr2 to the mPNP complex, together with the mRNA export cofactor, Yra1 (Iglesias et al., 2010). Nab2 also interacts with the NPC-associated protein Mlp1 to facilitate targeting of the mPNP complex to the pore (Fasken et al., 2008; Green et al., 2003; Vinciguerra et al., 2005). Following Mex67-mediated export through the NPC, Gfd1 helps tether Nab2 to the cytoplasmic face of the pore (Zheng et al., 2010), where Nab2, Mex67, and other RNA export factors are released from the mRNP by the RNA helicase Dbp5 (Kelly and Corbett, 2009). Consistent with a function for Nab2 as a global mRNA export factor, a recent analysis of Nab2-bound transcripts showed that Nab2 associates with most transcripts in budding yeast (Batisse et al., 2009). Besides

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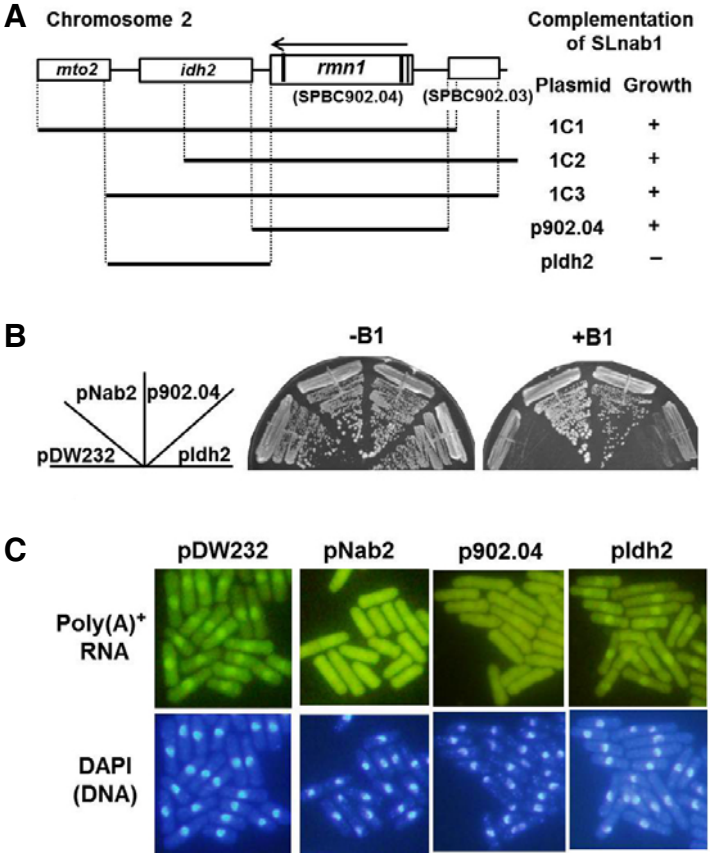

Fig. 1. (A) A schematic diagram represents the region surrounding the *rmn1* gene. Open boxes indicate the relative position and extent of the open reading frames. Plasmids that contain original clones and subcloned fragments are shown as horizontal bars. SLnab1 cells transfomed with different plasmids were spread on EMM agar in the presence of thiamine. Growth was monitored for 5 days at 30°C. +, normal growth; -, no growth. (B) Suppression of the growth defect in synthetic lethal mutant SLnab1 by *rmn1* and *spnab2*. SLnab1 cells carrying pDW232 (empty vector), pNab2 (*spnab2*), p902.04 (*rmn1*), and pIdh2 (*idh2*) were streaked onto EMM agar in the absence (-B1) and presence (+B1) of thiamine. (C) Poly(A)$^+$ RNA localization in SLnab1 carrying pDW232, pNab2, p902.04, and pIdh2. Cells were grown to the mid-log phase in appropriately supplemented EMM medium in the absence of thiamine (-B1) at 30°C. The cells were then shifted to EMM medium containing thiamine (+B1) and were grown for 18 h. Coincident DAPI staining is shown in the right panels.

linking mRNA 3′-end formation and mRNA export, Nab2 is involved in mRNA transcription, tRNA metabolism, and ribosomal subunit export (González-Aguilera et al., 2011). Thus, Nab2 is thought to be a central player in RNA metabolism in *S. cerevisiae*.

In contrast to *S. cerevisiae* Nab2 that is essential, the ortholog of the fission yeast *S. pombe* (spNab2) is dispensable for cell growth and mRNA export. However, spNab2 appears to also be involved in mRNA export, because spNab2 overexpression causes severe growth defects and intense accumulation of poly(A)$^+$ RNA in the nucleus (Yoon et al., 2009). To identify genes that genetically interact with *spnab2*, we isolated three mutants (SLnab1-3) that showed synthetic lethality under the repressed condition of *spnab2* expression. In this study, we describe the isolation of the novel *rmn1* gene as a multicopy suppressor that complements the growth defect in SLnab1. The *rmn1* gene is not essential for viability and mRNA export. However, its overexpression causes defects in growth and mRNA export, which is reminiscent of the *spnab2* phenotype. In addition, *Δrmn1Δspnab2* and *Δrmn1Δspmex67* double mutants exhibited synthetic lethality, and Rmn1p associated with Rae1p *in vivo*. Therefore, our data suggest that *rmn1* is a novel gene involved in mRNA export.

MATERIALS AND METHODS

Strains and culture

The *S. pombe* strains used in this study are listed in Table 1. Growth media and basic genetic and cell culture techniques for *S. pombe* have been described previously (Alfa, 1993; Moreno, 1991). Yeast extract plus supplement (YES) medium was used for general propagation of *S. pombe* cells and G418 (100 mg/L) was added to YES and Pombe glutamate (PMG) medium for selection of *kan*^{r} transformants. Appropriately supplemented Edinburgh minimal medium (EMM) was used to express genes from the *nmt1* promoter in the pREP3X and pREP81X plasmids (Basi et al., 1993). The *nmt1* promoter was repressed with 15 μM thiamine in EMM and PMG media (Forsburg, 1993). *Escherichia coli* TOP10 strain was used for plasmid propagation and selection.

Isolation of *rmn1* gene

SLnab1 synthetic lethal mutant cells were transformed with a partial Sau3A genomic library, which was cloned into the *Sal*I site of pUR18 (Barbet, 1992). We isolated the transformants that could grow on plates in the presence of thiamine at 28°C. The plasmids were rescued from these transformants, amplified in *E. coli*, and re-transformed into SLnab1 for confirmation. Nine plasmids complemented the SLnab1 growth defect in the presence of thiamine. The genomic clones in these plasmids were sequenced at both ends, and the DNA sequence was used to search the *S. pombe* genome database (http://www.sanger.ac.uk/Projects/S_pombe/, Sanger Center, UK). Five plasmids were eliminated from further investigation because they carried the *spnab2* gene. An overlapping region of the remaining three clones (two clones harbored identical insert DNA) contained one complete open reading frame (ORF), and this ORF was subcloned into pDW232 and transformed into SLnab1 to con-

Table 1. *S. pombe* strains used in this study

Strains	Genotype	Source
AY217	*h⁻ leu-32 ura4-D18*	Yoon et al. (1997)
SP286	*h⁺/h⁺ leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-210/ade6-216*	Matsumoto and Beach (1991)
SLnab1	*h⁻ leu-32 ura4-D18 h- leu1-32 ura4-d18 sln1-1 Pnmt-nab2-Tnmt::kan^r*	Park et al. (2012)
SP286 (*Δrmn1*)	*h⁺/h⁺ leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-210/ade6-216rmn1/Δrmn1::kan^r*	This study
AY217 (3X-Rmn1)	*h⁻ leu-32 ura4-D18 /* pREP3X-Rmn1	This study
Rmn1-GFP	*h⁻ leu-32 ura4-D18 rmn1-gfp:: ura4*	This study
Δrmn1	*h⁻ leu-32 ura4-D18 Δrmn1::kan^r*	This study
Δspnab2	*h⁺ leu-32 ura4-D18 Δspnab2::ura4*	This study
rae1-167	*h⁺ leu-32 ura4-D18 rae1-167*	Yoon et al. (1997)
Δspmex67	*h⁺ leu-32 ura4-D18 Δspmex67::ura4*	Yoon et al. (2000)
Δmlo3	*h⁺ leu-32 ura4-D18 Δmlo3::ura4*	Thakurta et al. (2005)

firm that it complemented the SLnab1 growth defect in the presence of thiamine (Fig. 1).

Construction of the *rmn1* null strain and plasmids

The *Δrmn1::kan^r* null mutation was constructed by double-joint polymerase chain reaction (PCR) (Yu et al., 2004). The total *rmn1* ORF was replaced by the maker gene *kan^r*. After the third round PCR, the amplified *Δrmn1::kan^r* fragment was purified and transformed into the SP286 diploid strain. G418-resistant transformants were selected and screened by PCR for disruption of one of the *rmn1* loci. The selected heterozygous diploid cells were sporulated, and ten tetrads were dissected.

An *rmn1* cDNA clone was made by reverse transcription (RT)-PCR from total RNA obtained from wild-type *S. pombe* cells and confirmed by DNA sequencing. The entire *rmn1* ORF was cloned into pREP3X, pREP81X, and pZA69U (Maundrell, 1993) by creating *Xho*I and *Bam*HI sites immediately upstream of the *rmn1* initiation codon and downstream of the stop codon, respectively, by PCR. The *Xho*I and *Bam*HI digested PCR products were then ligated into the vectors cut with *Xho*I and *Bam*HI to generate pREP3X-Rmn1, pREP81X-Rmn1, and pZU-Rmn1 plasmids, respectively. pHA-Rmn1 and pHA-Nab2 were constructed by inserting each ORFs into pSLF273 (Forsburg and Sherman, 1997).

***In situ* hybridization**

In situ hybridization was performed as described previously (Yoon et al., 2000). Oligo-$(dT)_{50}$ carrying an α-digoxygenin at the 3′ end was used as the hybridization probe. FITC-anti-digoxygenin Fab antibody (Roche Applied Science, Germany) was used for detecting the hybridization probe by fluorescence microscopy, and 4′,6-diamidino-2′-phenyindole (DAPI) was used for observing the DNA.

Semi-quantitative RT-PCR

Total RNA was purified from appropriate *S. pombe* cultures using TRIZOL reagent (Invitrogen, USA). One twentieth of the first-strand cDNA, synthesized from 1 μg total RNA using PrimeScript High Fidelity RT-PCR kit (TaKaRa Bio, Japan), was used as the PCR templates. The following primers were used: *rmn1* forward, AGTTCGGTGTATTGGAGAAAGTCG; *rmn1* reverse, GTCCTGAAGCGTAAGGATGATGAG; *rae1* forward, TCGTTGAAGCTATGGGTACTTCAC; *rae1* reverse, CTCCAGTACGATTAAACGTGCTGC. The PCR product was about 600 bp.

Immunoprecipitation and Western blot analyses

Immunoprecipitation and Western blot analyses were performed as described previously (Yoon et al., 2000). Cells transformed with pHA-Rmn1, pHA-Nab2, pREP4X-Rae1, or the empty vector were harvested after washing with stop buffer and resuspended in ice-cold lysis buffer containing 6 mM Na_2HPO_4, 4 mM NaH_2PO_4, 1% NP-40, 150 mM NaCl, 50 mM NaF, protease inhibitor cocktail (Sigma-Aldrich, USA), 4 μg/ml leupeptin, and 1 mM PMSF. Lysates were prepared by breaking the cells with glass beads using a Mini Beadbeater (Biospec, USA). Samples immunoprecipitated with the indicated antibodies and whole cell extracts were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. Polyclonal rabbit anti-Rae1 was described previously (Bharath et al., 1997). The other antibodies used in this study, monoclonal and polyclonal anti-HA (Santa Cruz Biotechnology, USA), anti-GFP (AbD Serotec, Germany), and IgG-conjugated protein A-agarose (Santa Cruz Biotechnology) were purchased from the manufacturers. HRP-conjugated secondary antibody were probed and developed with enhanced chemiluminescence (ECL) solution (Amersham, GE Healthcare, USA). Signals were detected and analyzed with the Kodak image station 4,000 MM PRO (Kodak, USA).

RESULTS AND DISCUSSION

Complementation of the synthetic lethal mutant SLnab1 growth defect by Rmn1

Synthetic lethality is the combination of two mutant genes that leads to cell death, but each single mutant gene separately does not cause cell death. Synthetic lethality can be used to identify genes whose products function in parallel redundant pathways or act in the same essential pathway (Dixon et al., 2008; Fabre and Hurt, 1997). To identify genes involved in mRNA biogenesis or mRNA export in *S. pombe*, we have performed a synthetic lethal screen to isolate mutants that are genetically linked to *spnab2* (Park and Yoon, 2012). We generated a parental strain to screen of synthetic lethal mutations, in which the chromosomal wild-type *spnab2* allele was replaced with the P_{nmt}-*spnab2* allele. In the P_{nmt}-*spnab2* allele, the *spnab2* ORF was fused to the weakest version of the *nmt1* promoter (P_{nmt}), which was subject to transcriptional repression by thiamine; thus, the *spnab2* gene was expressed under the control of the *nmt1* promoter instead of its own promoter. We isolated

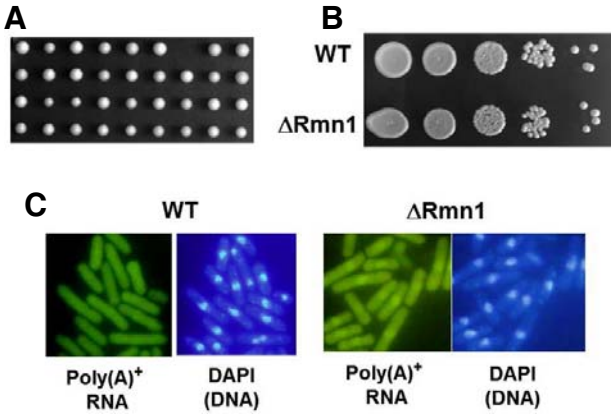

Fig. 2. The *Δrmn1* deletion mutants showed no growth defect but exhibited slight accumulation of Poly(A)$^+$ RNA in the nucleus. (A) Tetrad analysis of the h^+/h^{90} heterozygous diploids cells (*rmn1*$^+$/*Δrmn1*) after sporulation on ME plates. Nine tetrads were dissected on YES plates and were incubated for 4 days at 30°C. (B) Growth of haploid *rmn1*$^+$ (WT) cells and *Δrmn1* deletion mutant cells were monitored by spot assay on YES medium for 4 days at 30°C. (C) Poly(A)$^+$ RNA localization in *rmn1*$^+$ cells and *Δrmn1* deletion mutant cells. Cells were grown to the mid-log phase in EMM medium.

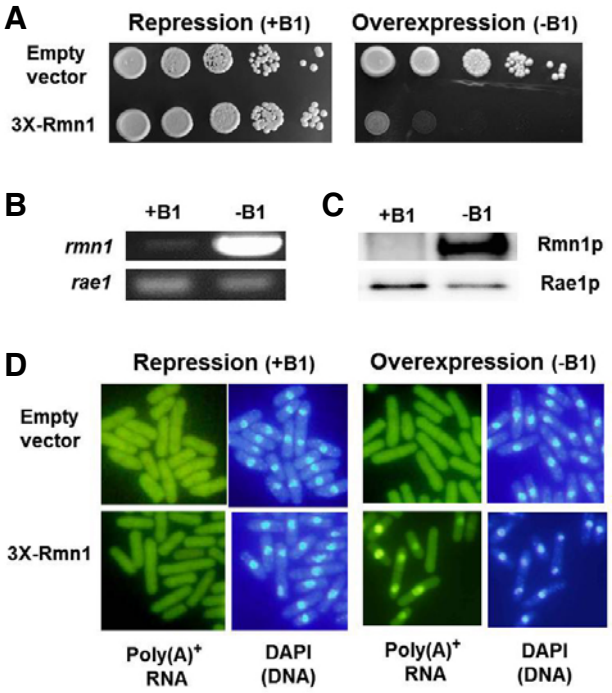

Fig. 3. Overexpression of *rmn1* causes growth inhibition accompanied by mRNA export defect. (A) Haploid wild-type (AY217) cells carrying the pREP3X plasmid (empty vector) or pREP3X-Rmn1 (3X-Rmn1) were monitored by spot assay for 4 days at 30°C in repressed or over-expressed conditions. (B) Semi-quantitative RT-PCR or (C) Western blot analyses were performed to assess *rmn1* expression in the presence (+B1) and absence of thiamine (-B1). *rae1* mRNA and the Rae1 protein were used as internal standards. (D) Poly(A)$^+$ RNA localization in cells carrying pREP3X plasmid (empty vector) or pREP3X-Rmn1 (3X-Rmn1). Cells were grown to the mid-log phase in appropriately supplemented EMM medium in the presence of thiamine at 30°C. The cells were then washed and shifted to EMM medium without thiamine (-B1) or with thiamine (+B1) and grown for 18 h. Coincident DAPI staining is shown in the right panels.

three synthetic lethal mutants (SLnab1-3) by random mutagenesis, which harbor the P_{nmt}-*spnab2* allele and a synthetic lethal mutant allele (*sln1-1*, *sln2-1*, or *sln3-1*) in unknown genes, respectively. In the case of the SLnab1 mutant, synthetic lethal cells remained viable when *spnab2* was expressed from the P_{nmt}-*spnab2* allele in the absence of thiamine (permissive condition that mimics *spnab2*$^+$ *sln1-1*), whereas they showed growth defects when *spnab2* expression was repressed by adding thiamine (synthetic lethal condition that mimics *Δspnab2 sln1-1*). All mutants also exhibited an accumulation of poly(A)$^+$ RNA in the nucleus under the synthetic lethal condition (Park and Yoon, 2012).

In this study, we isolated genomic clones from a partial *Sau*3A library through functional complementation of growth defects of SLnab1 mutants harboring the P_{nmt}-*spnab2* allele and the *sln1-1* synthetic lethal allele, as mentioned in "Materials and Methods". Three genomic clones (1C2, 1C2, and 1C3) were isolated, which allowed growth of SLnab1 cells when *spnab2* was repressed in the presence of thiamine. These genomic clones have an overlapping region, which contains only one complete ORF, SPBC902.04 (Fig. 1A). To confirm whether this ORF is capable of complementing SLnab1, two subclones (p902.04 and pIdh2) were constructed in the pDW232 vector and transformed into SLnab1 cells. As shown in Fig. 1A, p902.04 containing the whole SPBC902.04 ORF was able to restore the growth of SLnab1 under synthetic lethal conditions, similar to the pNab2 containing the *spnab2* gene. But, pIdh2, which carried the whole *idh2* gene next to the SPBC902.04 ORF, did not complement the SLnab1growth defect as expected (Fig. 1B). The SLnab1 mutant cells accumulated poly(A)$^+$ RNA in the nucleus under the synthetic lethal condition. To determine whether the SPBC902.04 gene also complemented the SLnab1-mRNA export defect, poly(A)$^+$ RNA distribution was examined in these cells grown under the synthetic lethal condition (+B1). As shown in Fig. 1C, poly(A)$^+$ RNA of the SLnab1 cells transformed with p902.04 or pNab2 was distributed throughout the whole cell, similar to that of wild-type cells, whereas pDW232 (empty vector) and pIdh2 did not complement the mRNA export defect of SLnab2 cells. These results raised the possibility that the SPBC902.04 gene might be involved in mRNA biogenesis and mRNA export similar to *spnab2*.

The SPBC902.04 ORF contained three introns and encodes a 589 amino-acid-long protein with a predicted molecular weight of 66.9 kDa and an isoelectric point (pI) of 6.65. We confirmed these introns by sequencing the cDNA obtained from total RNA (data not shown). A BLASTP search of the protein databases revealed that the hypothetical SPBC902.04 protein showed no significant sequence similarity to known proteins of other organisms or to *S. pombe* itself. However, the Pfam database identified one RRM in the central region (amino acids 244-308) of this protein. We named this *S. pombe* gene *rmn1* (gene encoding a RRM-containing protein, which is isolated from a synthetic lethal mutant with *spnab2*).

Because the *rmn1* gene was isolated by its ability to confer growth of SLnab1 (P_{nmt}-*spnab2 sln1-1*) cells in the presence of thiamine, we checked whether *rmn1* is a cognate synthetic lethal *sln1* gene or an extragenic multicopy suppressor gene. The DNA fragment spanning the *rmn1* gene was amplified by

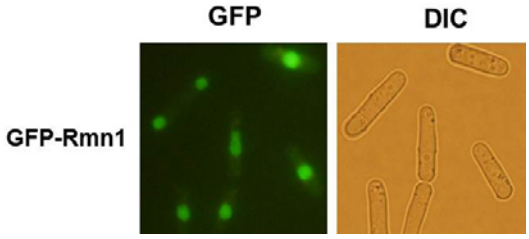

Fig. 4. Localization of Rmn1p fused to GFP. Cells were grown to the mid-log phase in appropriately supplemented EMM medium at 30°C. Coincident differntial interference contrast (DIC) images are also shown in the right panels.

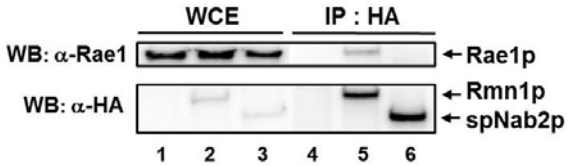

Fig. 5. Rmn1p associates with Rae1p. Lysates from *Δspnab2* cells transformed with pHA-Nab2 (lanes 3 and 6) and *Δrmn1* cells transformed with pHA-Rmn1 (lanes 2 and 5) and empty vector (lanes 1 and 4) were immunoprecipitated with anti-HA antibody. Immunoprecipitated samples (IP: HA) and whole cell extracts (WCE) were subjected to Western blotting to detect Rae1p, HA-Rmn1p, and HA-spNab2p.

PCR from SLnab1 cells and sequenced. The DNA sequence obtained was identical to that of wild-type *rmn1*. The plasmid containing the *rmn1* gene obtained from SLnab1 cells was still able to complement the SLnab1 growth defect. These results indicated that *rmn1* is not the *sln1* gene but a multicopy suppressor of the P_{nmt}-*spnab2 sln1-1* synthetic lethality. We could not find the *sln1* gene in this screen. A simple explanation for this failure is that our genomic DNA library might not cover the entire genome or that the size of *sln1* gene might be too big to be contained in this library, in which the average size of insert DNA fragments is 4-5 kb.

rmn1 gene deletion resulted in slight mRNA accumulation in the nucleus

To determine the *rmn1* knockout phenotype, a null mutant in the h^+/h^+ diploid strain was constructed by replacing one of the *rmn1* loci with a *kan*^{r} gene using a one-step gene disruption method. The diploids were allowed to sporulate, and ten tetrads were dissected. All haploid spores formed colonies (Fig. 2A), and the *kan*^{r} marker segregated at a ratio of 2:2. The haploid strain, with a deletion of *rmn1* (*Δrmn1::kan*^{r}), grew normally at temperatures of 18-37°C (Fig. 2B and data not shown). Thus, *rmn1* was not an essential gene for growth at any temperature. Poly(A)$^+$ RNA distribution was examined to determine whether deletion of the *rmn1* gene affected mRNA export. The *rmn1* knockout cells mildly accumulated poly(A)$^+$ RNA in the nucleus, wheras the poly(A)$^+$ RNA in the wild-type strain was distributed throughout the whole cell. These results demonstrate that loss of the Rmn1 protein had a slight impact on mRNA export in *S. pombe*. However, the defect of mRNA export in *Δrmn1* null cells seemed to be too mild to exert a deleterious effect on growth.

Overexpression of *rmn1* inhibits growth and mRNA export

Although the Rmn1 protein is not essential, if it is involved in the nuclear export of mRNA, its overexpression could inhibit nuclear export of poly(A)$^+$ RNA by interacting and titrating out essential mRNA export factors. Thus, we checked whether *rmn1* overexpression would affect growth and mRNA export. We transformed the wild-type strain with the pREP3X-Rmn1 plasmid, in which *rmn1* is highly expressed under the control of the strongest *nmt1* promoter in the absence of thiamine (Forsberg, 1993). We ensured that the amount of *rmn1* mRNA, detected by semi-quantitative RT-PCR, was highly elevated when the cells harboring pREP3X-Rmn1 were grown in the absence of thiamine (Fig. 3B). The amount of HA-Rmn1 fusion expressed from the same *nmt1* promoter was also markedly increased in the absence of thiamine (Fig. 3C). When *rmn1* was overexpressed in the pREP3X-Rmn1 plasmid, we tested if cell growth and nuclear export of poly(A)$^+$ RNA is inhibited. As shown in Fig. 3A, *rmn1* overexpression in the absence of thiamine (-B1) caused a severe growth defect. This growth inhibition was accompanied by intense accumulation of poly(A)$^+$ RNA in the nucleus (Fig. 3D). However, when *rmn1* expression from the plasmid was repressed in the presence of thiamine (+B1), cell growth and the distribution of poly(A)$^+$ RNA appeared normal as in wild-type cells. These results suggest that highly overexpressed Rmn1p likely interacted with and titrated out other proteins that are essential for growth and nuclear export of mRNA. On the other hand, the *spnab2* overexpression also showed a growth inhibition and mRNA export defect (Yoon, 2009) that was similar to the *rmn1* overexpression phenotypes.

The GFP-Rmn1 fusion protein is predominantly localized in the nucleus

To gain further insight into Rmn1p function, we determined the subcellular localization of Rmn1p tagged at the N or C terminus with GFP. Both fusion proteins were functional as they complemented the SLnab1growth defect and the mRNA export defect of *Δrmn1* cells (data not shown). An integrated version of the *rmn1-gfp* fusion was then constructed at the *rmn1* locus, and the localization of the fusion protein was determined. The Rmn1-GFP signal was predominantly concentrated in the nucleus (Fig. 4). This Rmn1p localization pattern was also similar to that of spNab2p.

The *Δrmn1* allele showed synthetic lethality in combination with *Δspnab2* and *Δspmex67*

The observation that *rmn1* functions as a multicopy suppressor of SLnab1 (P_{nmt}-*spnab2 sln1-1*) and overexpression and repression of *rmn1* and *spnab2* exhibited similar phenotypes suggested that Rmn1p and spNab2p may have parallel redundant function during mRNA export. To test this possibility, we determined if there was a genetic interaction between *Δrmn1* and *Δspnab2* in *S. pombe.* If we crossed the *Δrmn1* mutant cells with the *Δspnab2* mutant cells, we had a 25% chance of obtaining meiotic spores harboring *Δrmn1Δspnab2* double mutants and wild-type *rmn1*$^+$*spnab2*$^+$, respectively, because *spnab2* and *rmn1* genes are located on the different chromosomes. However, we failed to get the *Δrmn1Δspnab2* double mutant spores in this cross, wheras wild-type spores harboring *rmn1*$^+$ *spnab2*$^+$ were obtained according to probability. These results indicate that the combination of *Δrmn1* and *Δspnab2* results in synthetic lethality. The synthetic lethality of the *Δrmn1 Δspnab2* double mutants supported the possibility that the mRNA export function of both Rmn1 and spNab2 proteins may be redundant in *S. pombe*.

Next, we wanted to know if there was a functional relationship between Rmn1p and other mRNA export factors such as spMex67p, Rae1p, and Mlo3p. In contrast to *S. cerevisiae* Mex67p, the *S. pombe* ortholog (spMex67p) is not essential for normal mRNA export and growth (Yoon et al., 2000). Instead, nuclear pore associated Rae1p (*S. cerevisiae* Gle2p) functions as an essential mRNA export factor. The temperature sensitive *rae1-167* mutant rapidly accumulates poly(A) RNA in the nucleus when the cells are shifted to nonpermissive temperatures (Brown et al., 1995). The *S. pombe* Mlo3p (*S. cerevisiae* Yra1p), a putative RNA annealing protein, is functionally linked to Rae1p during mRNA export (Thakurta et al., 2005). We crossed the *Δrmn1* mutant cells with *rae1-167*, *Δspmex67*, and *Δmlo3* mutant alleles, respectively, to get double mutant spores. The *Δrmn1rae1-167* and *Δrmn1Δmlo3* double mutants exhibited no additive defect on growth and mRNA export (data not shown). However, the combination of *Δrmn1* with *Δspmex67* resulted in synthetic lethality. The simplest interpretation of these results is that Rmn1p might be involved in the Rae1-mediated mRNA export process.

Rmn1p associates with Rae1p in *S. pombe* extract

To investigate whether the observed genetic interactions represent a physical association with each other, we performed immunoprecipitations. Whole cell extracts were prepared from cells transformed with pHA-Nab2 and pZU-Rmn1 plasmids, which expressed GFP-Rmn1p and HA-tagged spNab2p from plasmids. GFP-Rmn1 proteins were immunoprecipitated with anti-GFP antibody followed by Western blotting with anti-HA antibody. However, we did not detect spNab2p co-immunoprecipitated with Rmn1p (data not shown). This result suggests that Rmn1p and spNab2p may not work simultaneously as components of the same complex.

Next, we determined whether Rmn1p or spNab2p are able to associate with Rae1p. Whole cell extracts prepared from cells transformed with pHA-Nab2, pHA-Rmn1, and empty vector, were immunoprecipitated with anti-HA and analyzed by Western blotting with antibodies against Rae1p. Rae1p was co-immunoprecipitated in cells expressing HA-Rmn1p, whereas no Rae1p was detected in cells carrying an empty vector or HA-spNab2p (Fig. 5). These results suggest that Rmn1p could interact with the Rae1p complex *in vivo*, but we could not rule out the possibility that this association would be mediated by mRNA.

The mechanisms and factors required for both mRNA processing and mRNA export are broadly conserved from yeast to humans with some differences in details among species. Although these are also conserved in budding and fission yeasts, there are some differences between them. In *S. cerevisiae*, the mRNA export receptor Mex67 is essentially required for the export of all mRNA classes. Additionally, its adaptors such as Yra1, Npl3, and Nab2, which recruit Mex67p to mRNA, are also essential for normal mRNA export (Stewart, 2010; Tutucci and Stutz, 2011). However, the *S. pombe* Mex67-mediated pathway is not essential but appears to play auxiliary roles for bulk mRNA export, because the *S. pombe* orthologs of Mex67, Nab2, and Yra1 are not essential for growth and mRNA export (Thakurta et al., 2005; Yoon et al., 2000; 2009). Instead, the Rae1-mediated pathway functions as an essential mRNA export pathway in this fission yeast (Brown et al., 1995; Thakurta et al., 2005). It is currently unclear whether different classes of mRNAs are exported *via* different export pathways, and whether a specific export pathway may be more important than others depending upon species. Therefore, it would be intriguing to compare the differences in the two yeasts, which would help to advance our understanding of mRNA export and its integration into the gene expression pathway.

In summary, we isolated a novel *rmn1* gene as a multicopy suppressor gene of a synthetic lethal *sln1-1 Δspnab2* mutant. We showed that *Δrmn1* caused synthetic lethality in combination with *Δspnab2* or *Δspmex67*. Moreover, *rmn1* overexpression inhibited growth and mRNA export, and Rmn1p co-immunoprecipitates with Rae1p. These results suggest that Rmn1p might be involved in the Rae1-mediated mRNA export pathway. Further molecular and cellular studies of Rmn1p will provide insight into the function and mechanism of this protein during mRNA biogenesis and export.

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