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NOTE

Isolation of Synthetic Lethal Mutations in the *rsm1*-null Mutant of Fission Yeast

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To identify mutations in genes that are genetically linked to *rsm1*, we performed a synthetic lethal genetic screen in the fission yeast, *Schizosaccharomyces pombe*. Four mutations that showed synthetic lethality in combination with the *rsm1*null allele were isolated from approximately 320,000 colonies and defined in three complementation groups. One mutant (SLrsm1) exhibited a significant accumulation of poly(A)$^+$ RNA in the nucleus under synthetic lethal conditions, while the rest had no mRNA export defects. In addition, some genes (*spmex67*, *rae1*, or *mlo3*) required for mRNA export complemented the growth defects of the identified mutants. These results suggest that the isolated mutants contain mutations in genes that are involved in mRNA export and/or pre-mRNA retention.

***Keywords*:** *rsm1*, synthetic lethality, mRNA export, *S. pombe*

During eukaryotic gene expression, mRNAs that are transcribed in the nucleus must be exported to the cytoplasm for translation. As transcription progresses, the nascent transcript emerging from RNA polymerase II is successively processed to produce a mature mRNA and is packaged into the messenger ribonucleoprotein (mRNP) complex. The export of mRNA from the nucleus is a complex process that is interconnected with transcription, three main mRNA processing event (5'-end capping, splicing, and 3'-end cleavage followed by polyadenylation), and mRNA quality control for retention and/or degradation of aberrant mRNAs in the nucleus (Saguez *et al*., 2005; Köhler and Hurt, 2007; Fasken and Corbett, 2009; Rondón *et al*., 2010).

For the formation of export-competent mRNP, evolutionally conserved mRNP assembly factors are co-transcriptionally recruited to maturing transcripts. These factors include the TREX complex [THO complex plus mRNA export factors, such as RNA helicase, Sub2 (in *Saccharomyces cerevisiae*)/ UAP56 (in metazoan), and mRNA binding protein, Yra1/ ALY], heterodimeric mRNA export receptor Mex67-Mtr2/ NXF1-p15, poly(A) RNA-binding protein Nab2, heterogeneous nuclear ribonucleoproteins (hnRNPs), such as Npl3, and the THSC/TREX-2 complex (Rodriguez *et al*., 2004; Rougemaille *et al*., 2008; Rondón *et al*., 2010). The export-competent mRNPs are then targeted to, and translocated through, the nuclear pore complexes (NPCs), which are huge assemblies inserted in the nuclear envelope and function as the sole gates for macromolecular traffic between the nucleus and the cytoplasm (Tran and Wente, 2006). Export of the mRNPs through NPCs is mediated primarily by weak interactions between the export factors and nucleoporins (proteins composing NPC) lining the central transport channels of the NPCs (Rodriguez *et al*., 2004; Cole and Scarcelli, 2006). To release mRNAs for translation at the cypoplasmic face of the NPC, exported mRNPs are remodeled by DEAD-box RNA helicase Dbp5, which has ATPase activity that is activated by Gle1 and further stimulated by the small molecule, inositol hexakiphosphate (IP_6) (Alcázar-Román *et al*., 2006; Weirich *et al*., 2006).

On the other hand, eukaryotic cells have mRNA quality control mechanisms that recognize and degrade improperly processed or incorrectly packaged mRNAs, which may impair mRNA metabolism and generate harmful proteins. Nuclear mRNA quality control mechanisms rapidly degrade aberrant mRNAs by the exosome and retain aberrant mRNAs near the transcription sites or at the NPCs (Schimid and Jensen, 2008; Fasken and Corbett, 2009). In *S. cerevisiae*, unspliced pre-mRNAs are retained at the nuclear pore, preventing their export to the cytoplasm, by the nuclear pore-associated proteins, Mlp1 and Pml39, the nucleoporin Nup60, the nuclear envelope protein Esc1, and the pre-mRNA retention and splicing (RES) complex protein Pml1 (Dziembowski *et al*., 2004; Galy *et al*., 2004; Palancade *et al*., 2005; Lewis *et al*., 2007).

In *S. pombe*, Rae1 was first identified as an mRNA export factor (Brown *et al*., 1995). Subsequently, the *S. pombe* ortholog of mRNA receptor Mex67 was isolated as a multicopy suppressor of a temperature sensitive mutation of *rae1* that showed an mRNA export defect (Yoon *et al*., 2000). In addition, *rsm1* was isolated from synthetic lethal genetic screen with a *mex67* null allele (Yoon, 2004). Nonessential Rsm1 is involved in mRNA export and shares homology with the nuclear pore-associated protein Pml39, which is involved in pre-mRNA retention.

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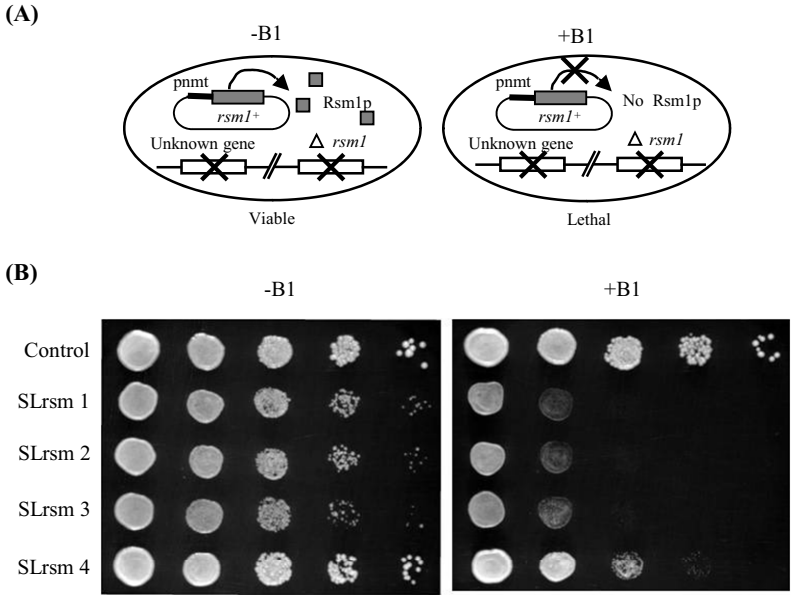

Fig. 1. Isolation of mutants that are synthetic lethal with the Δ*rsm1* null allele. (A) Schematic diagram of synthetic lethal screen. Wild-type *rsm1*$^+$ gene is denoted by shaded boxes and expressed under the control of the *nm1* promoter (pnmt). The Δ*rsm1* null allele and unknown mutated genes are shown by open boxes with a cross. Synthetic lethal mutants are kept viable by expression of *rsm1*$^+$ from a pREP81X plasmid in the absence of thiamine (-B1). In the presence of thiamine (+B1), the expression of *rsm1*$^+$ is repressed, resulting in the inhibition of growth. (B) Growth of synthetic lethal mutants. Control strain (Δ*rsm1*/81X-Rsm1) and synthetic lethal mutants (SLrsm1 through SLrsm4) were grown to saturation, serially diluted in 10-fold dilutions, and spotted onto EMM agar in the absence (-B1) and the presence (+B1) of thiamine. Cells were incubated for 4 days at 28°C.

In this study, we describe the isolation of synthetic lethal mutations in combination with the *rsm1* null allele. The basic genetic and cell culture techniques used in this work have been described (Moreno *et al*., 1991; Alfa *et al*., 1993). Yeast extract plus supplement (YES) medium was used for general propagation of *S. pombe* cells and appropriately supplemented EMM medium was used to express genes from the *nmt* promoter of the pREP81X plasmid (Basi *et al*., 1993; Maundrell, 1993). The *nmt1* promoter was repressed by 15 μM thiamine in EMM medium (Forsburg, 1993).

Synthetic lethal screening is one of the most successful genetic approaches in yeast for uncovering the hidden relationships or functions of nonessential genes (Fabre and Hurt, 1997; Dixon *et al*., 2008). Synthetic lethality is described as the death of a cell that contains two mutations in combination, whereas a cell with either individual mutation can survive. Synthetic lethality can occur when two genes function in parallel redundant pathways or act in the same essential pathway. Therefore, synthetic lethal screening was used to identify functionally overlapping components that do not physically interact with the Rsm1 protein, as well as physically interacting components of Rsm1.

For this screen, we used the Δ*rsm1* deletion allele, in which the entire *rsm1* gene is replaced with the *kan*^{r} gene (Kang and Yoon, 2008) for antibiotic G418 resistance. The Δ*rsm1* null mutant is viable, but it has a slight nuclear poly(A)$^+$ RNA accumulation phenotype (Yoon, 2004). To identify mutations that are lethal in combination with the Δ*rsm1* allele, we used Δ*rsm1*/81X-Rsm1 cells (*h*$^-$ *leu1-32 ura4-d18* Δ*rsm1::kan*^{r}/pREP81X-Rsm1) as a parental strain, which harbors the pREP81X-Rsm1 plasmid. The *rsm1* in pREP81X-Rsm1 is expressed from the thiamine-repressible *nmt1* promoter (the weakest version) in the absence of thiamine (vitamin B1), but not in the presence of thiamine (Fig. 1A). This strain was mutagenized with 3% ethyl methanesulfonate (EMS) at 24°C for 3 h, resulting in a 30% survival rate. After washing three times with EMM broth, the cells were spread on EMM agar plates in the absence of thiamine, and replica-plated onto EMM agar plates in the presence of thiamine. Approximately 320,000 colonies were screened for the inability to grow in the presence of thiamine. Identification of the synthetic lethal mutants was aided by the use of the dye phloxin B, which is taken up and accumulated in dead cells, causing the dead colonies to turn red. The parental strain and mutants lacking a synthetic lethal mutation were able to grow whether Rsm1p was expressed from the plasmid or not (Fig. 1B), because the *rsm1* gene is not essential for growth (Yoon, 2004). Potential synthetic lethal mutants were identified as tiny red colonies in the presence of thiamine (expression of Rsm1p from the plasmid is repressed) and big pink colonies on plates lacking thiamine (Rsm1p from the plasmid is expressed). The synthetic lethal phenotype of these mutants was confirmed on EMM plates with and without thiamine. Fourteen candidates that were defective for growth specifically in the presence of thiamine were isolated from the first screen (data not shown).

Next, we wanted to see whether the growth defects of the mutants in the presence of thiamine were due to synthetic lethality (i.e., the lack of Rsm1 protein) or some other unknown reason. For this purpose, the screened mutants were transformed with a plasmid (pRsm1) bearing the genomic

rsm1$^+$ gene whose expression is under the control of its own promoter and is not affected by thiamine. If the mutants harbor a true synthetic lethal mutation with the Δ*rsm1* allele, transformation of pRsm1 will rescue the growth defects of the mutants in the presence of thiamine, because Rsm1 protein is expressed from the plasmid (pRsm1) even when Rsm1 expression from the *nmt* promoter on the pREP81X-Rsm1 plasmid is repressed by thiamine. We found that 11 out of 14 candidates were rescued by the pRsm1 plasmid, indicating that three candidates were not synthetic lethal mutants and were excluded from subsequent experiments. As expected, transformation with empty pDW232 vector did not complement the grow defects of any of the mutants in the presence of thiamine.

The 11 candidates were backcrossed to the Δ*rsm1*/81X-Rsm1 strain (*h*$^+$ *leu1-32 ura4-d18* Δ*rsm1::kan*^{r} /pREP81X-Rsm1) in order to clean up background mutations. Among the 11 candidates, eight mutants were successfully crossed and produced spores that gave rise to colonies retaining the growth defect phenotype. These eight mutants were tested to determine whether the synthetic lethality with Δ*rsm1* was due to a single mutation locus. To separate the synthetic lethal mutations, eight mutants were crossed with an *rsm1*$^+$ strain (AY216; *h*$^+$ *leu1-32 ura4-d18*, or AY217; *h*$^-$ *leu1-32 ura4-d18*) and random spore analysis was performed. The resulting spores should give rise to four different types of colonies, two parental types and two recombinant types. One parental type showed the original synthetic lethal phenotype and the other was wild type. Recombinant colonies with only the *rsm1::kan*^{r} allele grew on media containing the antibiotic, G418. The other recombinant type harboring only a synthetic lethal mutation should be viable. When the colonies assumed to harbor only the synthetic lethal mutation were crossed again to a Δ*rsm1*/81X-Rsm1 strain, we expected to obtain spores that give rise to colonies with synthetic lethal phenotypes identical to those of the original mutants. In 4 of the 8 candidates, we obtained spores that showed synthetic growth defects. This demonstrated that neither synthetic lethal mutation alone affected cell viability, but conferred cell death only in combination with Δ*rsm1*. This result also indicated that the synthetic lethality was due to a single locus. The four synthetic lethal mutants confirmed as described above were named as SLrsm1 through SLrsm4, respectively (Fig. 1B).

To define the complementation groups, we set up crosses among the synthetic lethal mutants. If two mutations fell in the same complementation group, no wild-type spores were produced. Except for a cross between SLrsm2 and SLrsm4, none of the crosses reproduced the synthetic lethal phenotype (i.e., wild type). This result revealed that SLrsm1, SLrsm2, and SLrsm3 fall in separate complementation groups, while SLrsm2 and SLrsm4 form a single complementation group.

To determine whether the growth defects of the synthetic lethal mutations were associated with mRNA export defects, the poly(A)$^+$ RNA distribution was examined in the SLrsm1, 2 , 3, and 4 mutants grown under permissive and restrictive conditions. *In situ* hybridization was performed, as previously described (Yoon, 2000). Oligo-$(dT)_{50}$ tagged with an α-digoxygenin at the 3′ end was used as the hybridization probe. FITC-anti-digoxygenin Fab antibody (Roche Applied Science, Germany) was used to detect the hybridized probe by fluorescence microscopy. The poly(A)$^+$ RNA of parental strain, Δ*rsm1*/81X-Rsm1 strain (control), was distributed throughout the entire cell whether grown in the presence or absence of thiamine (Fig. 2). SLrsm2, SLrsm3, and SLrsm4 did not show significant poly(A)$^+$ RNA accumulation in the nucleus, even in the synthetic lethal condition (+B1). These results suggest that synthetic lethality in these mutants is not associated directly with the defects of bulk mRNA export from the nucleus to cytoplasm. However, most cells of the SLrsm1 mutant had extensive poly(A)$^+$ RNA accumulation in the nucleus in the synthetic lethal condition (Fig. 2), suggesting that *rsm1* and the mutated gene in SLrsm1 genetically interact to affect the export of poly(A)$^+$ RNA out of the nucleus.

In *S. pombe*, several genes known to be involved in mRNA export have been isolated. In contrast to *S. cerevisiae MEX67*, which is essential for mRNA export, *mex67* of *S. pombe* (*spmex67*) is not required for normal mRNA export, although it is involved in mRNA export (Yoon *et al*., 2000). Instead, *rae1* is essential for mRNA export (Brown *et al*., 1995). Using systematic genetic screens, several genes that display synthetic lethal interactions with the temperature-sensitive *rae1-167* or Δ*spmex67* allele have been identified in *S. pombe*. These genes include soluble mRNA export factors and nucleoporins that

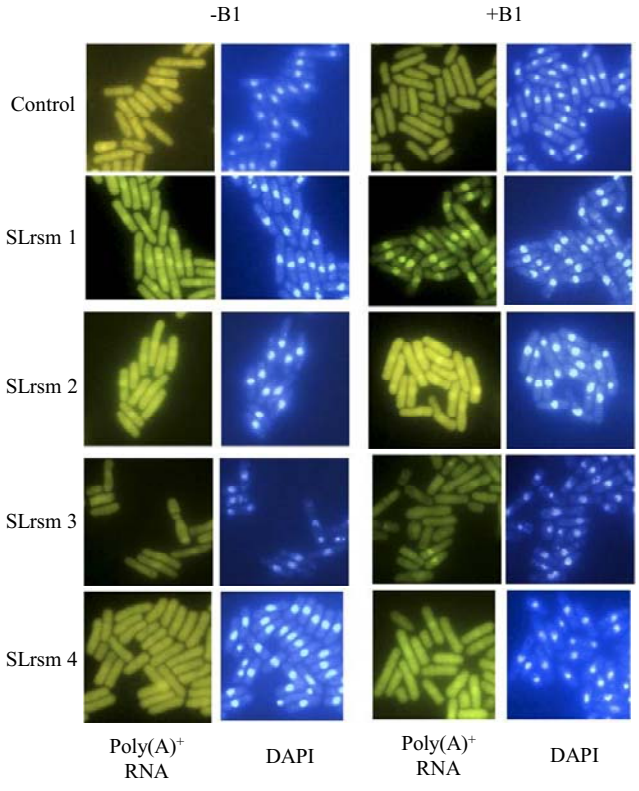

Fig. 2. Poly(A)$^+$ RNA localization in synthetic lethal mutants. Control strain (Δ*rsm1*/81X-Rsm1) and synthetic lethal mutants (SLrsm1 through SLrsm4) were grown to mid-log phase in appropriately supplemented EMM medium in the absence of thiamine (-B1) at 28°C. Cells were then shifted to EMM medium containing thiamine (+B1) and grown for 18 h. Coincident DAPI staining used to detect DNA is shown in the right panels.

Table 1. Complementation test of Synthetic lethal mutants by the genes known to be involved in mRNA export in *S. pombe*

Plasmids \ Strains	SLrsm1	SLrsm2	SLrsm3
pDW232	−	−	−
pRsm1	+	+	+
pMex67	+	+	+
pRae1	−	±	+
pMlo3	+	−	−
pNup184	−	−	−
pNpp106	−	−	−
pNup97	−	−	−

are involved in mRNA export (Yoon *et al*., 1997, 2000; Kozak *et al*., 2002; Yoon, 2004; Thakurta *et al*., 2005). Therefore, we wanted to know whether these genes could complement growth defects of the synthetic lethal mutations in SLrsm1-3. For this purpose, the synthetic lethal mutants were transformed with the empty vector, pDW232, or the plasmids bearing the genomic *spmex67*, *rae1*, *mlo3*, *npp106*, *nup184*, and *nup97* genes, respectively. As shown in Table 1, pDW232 was unable to rescue the phenotype, while pRsm1 harboring genomic *rsm1* complemented the growth defects of the mutants in the presence of thiamine. Interestingly, expression of the *spmex67* gene complemented the synthetic lethality of all mutants, and expression of the *rae1* gene rescued the lethality in SLrsm3 and partially in SLrsm2. In addition, expression of the *mlo3* gene suppressed the defects of SLrsm1. These results suggest that these genes act in parallel redundant pathways or in the same essential pathway, along with *rsm1* and the mutant gene. In contrast, *npp106*, *nup184*, and *nup97* genes, which encode nucleoporins, failed to complement the growth defects of SLrsm1-3.

In summary, we have isolated four mutations that are synthetically lethal when combined with the *rsm1* null mutation, one of which causes a defect in mRNA export. Because Rsm1 is involved in mRNA export (Yoon, 2004) and shares homology with *S. cerevisiae* Pml39 that operates in the pre-mRNA retention pathway (Palancade *et al*., 2005), it will be interesting to isolate the cognate synthetic lethal genes and multicopy suppressor genes in these mutants and to figure out the functions of these genes in mRNA export and pre-mRNA retention at the nuclear pore. These future studies will expand our understanding of mechanisms of the mRNA export and pre-mRNA retention pathways.

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References

Alcázar-Román, A.R., E.J. Tran, S. Guo, and S.R. Wente. 2006. Inositol hexakisphosphate and Gle1 activate the DEAD-box protein Dbp5 for nuclear mRNA export. *Nat. Cell Biol.* 8, 711-716.

Alfa, C., P. Fantes, J. Hyams, M. Mcleod, and E. Warbrick. 1993. Experiments with Fission Yeast. Cold Spring Harbor, Cold Spring Harbor Laboratory, New York, N.Y., USA.

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.

Brown, J.A., A. Bharathi, A. Ghosh, W. Whalen, E. Fitzgerald, and R. Dhar. 1995. A mutation in the *Schizosaccharomyces pombe rae1* gene causes defects in poly(A)$^+$ RNA export and in the cytoskeleton. *J. Biol. Chem*. 270, 7411-7419.

Cole, C.N. and J.J. Scarcelli. 2006. Unravelling mRNA export. *Nat. Cell Biol.* 8, 645-647.

Dixon, S.J., Y. Fedyshyn, J.L. Koh, T.S. Prasad, C. Chahwan, G. Chua, K. Toufighi, and *et al*. 2008. Significant conservation of synthetic lethal genetic interaction networks between distantly related eukaryotes. *Proc. Natl. Acad. Sci. USA* 105, 16653-16658.

Dziembowski, A., A.P. Ventura, B. Rutz, F. Caspary, C. Faux, F. Halgand, O. Laprévote, and B. Séraphin. 2004. Proteomic analysis identifies a new complex required for nuclear pre-mRNA retention and splicing. *EMBO J*. 23, 4847-4856.

Fabre, E. and E. Hurt. 1997. Yeast genetics to dissect the nuclear pore complex and nucleocytoplasmic trafficking. *Annu. Rev. Genet*. 31, 277-313.

Fasken, M.B. and A.H. Corbett. 2009. Mechanisms of nuclear mRNA quality control. *RNA Biol*. 6, 237-241.

Forsburg, S.L. 1993. Comparison of *Schizosaccharomyces pombe* expression systems. *Nucleic Acids Res*. 21, 2955-2956.

Galy, V., O. Gadal, M. Fromont-Racine, A. Romano, A. Jacquier, and U. Nehrbass. 2004. Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1. *Cell* 116, 63-73.

Kang, S. and J.H. Yoon. 2008. Genetic analysis of fission yeast *rsm1* which is involved in mRNA Export. *Kor. J. Microbiol*. 44, 98-104.

Köhler, A. and E. Hurt. 2007. Exporting RNA from the nucleus to the cytoplasm. *Nat. Rev. Mol. Cell Biol.* 8, 761-773.

Kozak, L., G. Gopal, J.H. Yoon, Z.E. Sauna, S.V. Ambudkar, A.G. Thakurta, and R. Dhar. 2002. Elf1p, a member of the ABC class of ATPases, functions as a mRNA export factor in *Schizosacchromyces pombe. J. Biol. Chem*. 277, 33580-33589.

Lewis, A., R. Felberbaum, and M. Hochstrasser. 2007. A nuclear envelope protein linking nuclear pore basket assembly, SUMO protease regulation, and mRNA surveillance. *J. Cell. Biol*. 178, 813-827.

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

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

Palancade, B., M. Zuccolo, S. Loeillet, A. Nicolas, and V. Doye. 2005. Pml39, a novel protein of the nuclear periphery required for nuclear retention of improper messenger ribonucleoparticles. *Mol. Biol. Cell* 16, 5258-5268.

Rodriguez, M.S., C. Dargemont, and F. Stutz. 2004. Nuclear export of RNA. *Biol. Cell* 96, 639-655.

Rondón, A.G., S. Jimeno, and A. Aguilera. 2010. The interface between transcription and mRNP export: From THO to THSC/TREX-2. *Biochim. Biophys. Acta*. 1799, 533-538.

Rougemaille, M., T. Villa, R.K. Gudipati, and D. Libri. 2008. mRNA journey to the cytoplasm: attire required. *Biol. Cell* 100, 327-342.

Saguez, C., J.R. Olesen, and T.H. Jensen. 2005. Formation of export-competent mRNP: escaping nuclear destruction. *Curr. Opin. Cell Biol*. 17, 287-293.

Schmid, M. and T.H. Jensen. 2008. The exosome: a multipurpose RNA-decay machine. *Trends Biochem. Sci*. 33, 501-510.

Thakurta, A.G., G. Gopal, J.H. Yoon, L. Kozak, and R. Dhar. 2005. Homolog of BRCA2-interacting Dss1p and Uap56p link Mlo3p and Rae1p for mRNA export in fission yeast. *EMBO J.* 24, 2512-

2523.

Tran, E.J. and S.R. Wente. 2006. Dynamic nuclear pore complexes: life on the edge. *Cell* 125, 1041-1053.

Weirich, C.S., J.P. Erzberger, J.S. Flick, J.M. Berger, J. Thorner, and K. Weis. 2006. Activation of the DExD/H-box protein Dbp5 by the nuclear-pore protein Gle1 and its coactivator InsP6 is required for mRNA export. *Nat. Cell Biol.* 8, 668-676.

Yoon, J.H. 2004. *Schizosaccharomyces pombe rsm1* genetically interacts with *spmex67*, which is involved in mRNA export. *J. Microbiol*. 42, 32-36.

Yoon, J.H., D.C. Love, A. Guhathakurta, J.A. Hanover, and R. Dhar. 2000. Mex67p of *Schizosaccharomyces pombe* interacts with Rae1p in mediating mRNA export. *Mol. Cell. Biol.* 20, 8767-8782.

Yoon, J.H., W.A. Whalen, A. Bharathi, R. Shen, and R. Dhar. 1997. Npp106p, a *Schizosaccharomyces pombe* nucleoporin similar to *Saccharomyces cerevisiae* Nic96p, functionally interacts with Rae1p in mRNA export. *Mol. Cell. Biol*. 17, 7047-7060.