Schizosaccharomyces pombe nup97, which Genetically Interacts with mex67, is Essential for Growth and Involved in mRNA Export

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We have isolated previously three synthetic lethal mutants in Schizosaccharomyces pombe, which genetically interact with mex67, in order to identify the genes involved in mRNA export. A novel nup97 gene was isolated by complementation of the growth defect in one of the synthetic lethal mutants, SLMex3. The nup97 gene contains one intron and encodes an 851 amino-acid protein that is similar to nucleoporins, Npp106p in S. pombe and Nic96p in Saccharomyces cerevisiae. The nup97 gene is essential for vegetative growth, and nup97 null mutant harboring pREP41X-Nup97 showed poly(A)$^+$ RNA export defect when expression of nup97 is repressed in the presence of thiamine. These results suggest that nup97 is involved in mRNA export from the nucleus to cytoplasm.

Keywords: nup97, mex67, synthetic lethality, mRNA export, Schizosaccharomyces pombe

Translocation of molecules between the nucleus and the cytoplasm plays important roles in eukaryotic cell functions. The sole gate for selective transfer of macromolecules between these compartments is the nuclear pore complex (NPC), which is embedded in the nuclear envelope that separates the nucleus from the cytoplasm. The NPC is a huge macromolecular assembly of about 40 and 60 Mda in mass in yeast and vertebrates, respectively (Rout et al., 2000; Cronshaw et al., 2002). Despite the difference in size, the NPC of yeast and metazoan is conserved in basic overall architecture, which consists of the central core, the cytoplasmic filaments, and the nuclear basket (Stoffler et al., 1999; Suntharalingam and Wente, 2003). The NPC is made up of approximately 30 different proteins termed collectively nucleoporins (Nups) (Rout and Aitchison, 2001; Vasu and Forbes, 2001). A recent direct structural and computational analysis revealed the modularity of the NPC architecture and the simplicity of NPC composition (Rout et al., 2000; Devos et al., 2004, 2006). The Nups are divided into three groups based on the predicted structural fold types (Devos et al., 2006). The transmembrane group containing transmembrane helices and cadherin fold may help to anchor the NPC in the nuclear envelope. The peripheral FG group containing FG repeats and coiled-coil fold lines the channel of the NPC and may be directly implicated in active nuclear transport to provide interaction regions for transport receptors. The central scaffold group containing β-propeller and α-solenoid folds may connect the two groups to form a complete NPC.

The export of mRNA is more complex than the nucleocytoplasmic transport of protein and other RNAs, because mature mRNAs are exported as huge ribonucleoprotein (RNP) complex, which is intimately required to remodeling of mRNP complex to pass through the NPC, and because the export of mature mRNA is tightly coupled to several steps of gene expression including transcription, splicing, and polyadenylation (Reed and Hurt, 2002; Cole and Scarcelli, 2006). Besides some specific Nups, a lot of soluble factors are involved in the mRNA export (Erkmann and Kutay, 2004). Although it is unclear how the only mature mRNPs are selected to export, binding of mRNA receptors to mRNA is necessary for export. The heterodimeric mRNA receptors, Mex67-Mtr2 in yeast and TAP/NXF-p15/NXT in metazoan, are recruited to mRNA by adaptor proteins such as Sub2/REF, EJC components, SR proteins, or THO/TREX components (Segref et al., 1997; Huang and Steitz, 2005; Moore, 2005; Gwizdek et al., 2006). Translocation of mRNP complex through NPCs appears to be facilitated by weak interaction between the heterodimeric mRNA receptors and FG-Nups (Braun et al., 2002; Rodriguez et al., 2004).

In Schizosaccharomyces pombe (S. pombe), mex67 (spmex67) is not essential, but is also involved in mRNA export (Yoon et al., 2000). The spmex67 gene was isolated as a multicopy suppressor of rae1-167 nup184-1 synthetic lethality and is also able to partially suppress a temperature sensitive mutation of rae1 that is essential for growth and mRNA export (Brown et al., 1995; Yoon et al., 2000). And spmex67 null allele is synthetically lethal with mutant alleles of some Nups such as npp106 and nup184, and with those of some soluble mRNA export factors such as dss1, elf1, mlo3, rae1, and rsm1 (Yoon et al., 1997, 2000; Whalen et al., 1999; Kozak et al., 2002; Yoon, 2004; Thakurta et al., 2005). In order to identify mutations in more genes that are functionally linked to mex67 in mRNA export, synthetic lethal mutant screen was performed previously and obtained three mutants that exhibited an accumulation of poly(A)$^+$ RNA in the nucleus under synthetic lethal conditions (Yoon, 2003).

In this study, we described the isolation of the novel nup97 gene by complementation of the growth defect in one of the

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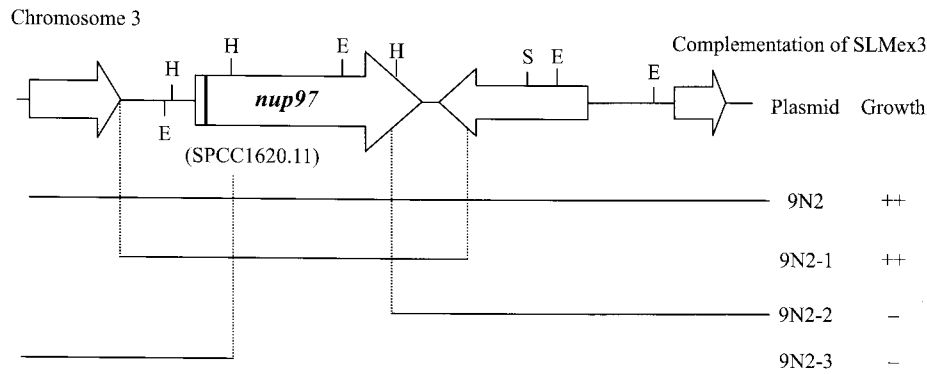(Tel) 82-2-920-7675; (Fax) 82-2-920-7675
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Fig. 1. Complementaion of growth defects of SLMex3 and a restriction map of the region surrounding the *nup97* gene. An open arrow indicates the extent and direction of open reading frame of the *nup97* and the other genes. The original clone and subcloned fragments, which were tested for the complementation of SLMex3, are shown as horizontal bars. SLMex3 cells carrying pREP81X-Mex67 were transfomed with different plasmids and were spread on EMM agar in the presence of thiamine. Growth was monitored for 5 days. + +, normal growth; –, no growth. E, *Eco*RI; H, *Hind*III; S, *Sal*I.

synthetic lethal mutants, SLMex3. In addition, the *nup97* deletion mutants were shown to cause defects in both growth and mRNA export. These results suggested that *nup97* is involved in the mRNA export in *S. pombe*.

Materials and Methods

Strains and culture

The *S. pombe* strains used were of wild type AY217: *h⁻ leu1-32 ura4-d18*; SP286: *h⁺/ h⁺ leu1-32/leu1-32 ura4-d18/ ura4-d18 ade6-210/ ade6-216* (Matsumoto and Beach, 1991), SLMex3: *h⁻ leu1-32 ura4-d18 slmex3 Δmex67::kanʳ* /pREP81X-Mex67*(Yoon, 2003), and Δnup97:*h⁻ leu1-32 ura4-d18 Δnup97:: ura4*/pREP41X-Nup97 (this work). The basic genetic and cell culture techniques used have been described (Moreno *et al.*, 1991; Alfa *et al.*, 1993). Appropriately supplemented EMM medium was used to express genes from the pREP plasmids containing the *nmt* promoter (Basi *et al.*, 1993; Maundrell, 1993). The *nmt* promoter was repressed by the addition of 15 µM thiamine in EMM medium (Forsburg, 1993).

Isolation of nup97 gene

SLMex3 cells (*h⁻ leu1-32 ura4-d18 slmex3 Δmex67::kanʳ* /pREP81X-Mex67) were transformed with a partial *Sau*3A genomic library that was cloned into the *Sal*I site of pUR18 (Barbet, 1992). Four transformants were isolated, which could grow on plates in the presence of thiamine at 28°C. The plasmids from four transformants were rescued into *Escherichia coli* and re-transformed into SLMex3 for confirmation. Three among four plasmids could complement again the growth defect of SLMex3 in the presence of thiamine. The genomic clones in three 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). Two plasmids were eliminated from further investigation because they carried the *mex67*gene. The remaining one clone contained four open reading frames (ORFs) and each gene was subcloned into pDW232 and transformed into SLMex3 to inves-

tigate which one can complement the growth defect of SLMex3 in the presence of thiamine (Fig. 1).

Construction of nup97 null mutants

The Δnup97:: *ura4⁺* null mutation was constructed by double-joint PCR (Yu *et al.*, 2004). Total *nup97* ORF except for 7 amino acids at carboxyl terminus was replaced by maker gene, *ura4⁺*. The primers for construction of Δnup97:: *ura4⁺* fragment is as follows. 5'-For; TGCTCAGTTGTGGAAAGC TG, 5'-Rev; ACATATAGCCAGTGGGATTTGTAGCTAATG GTCAAGATGTAAACAAGCAC, 3'-For; GGTGTTGGAAC AGAATAAATTAGATGATACTGCTGCTAAACAGGCTGA TG, 3'-Rev; TAGTTTAGACTACTCATCTC, 5'-Nest; CTCA TGATGCTGAATCAATTC, 3'-Nest; ACGCGATTCCTACA AGTCTC. After third round PCR, the amplified Δnup97:: *ura4⁺* fragment was gel-purified and was transformed into the SP286 diploid strain. Ura⁺ transformants were selected and screened by PCR for the disruption of one of the *nup97* loci. The strain was sporulated, and ten tetrads were dissected. Only two spores in a tetrad formed colonies and all viable cells showed *ura⁻* phenotype indicative of wild-type *nup97* allele (Fig. 2).

Because *nup97* gene is essential for vegetative growth, we used a haploid strain AY217 with pREP41X-Nup97 plasmid in which *nup97* expression is under the control of *nmt* promoter. This strain was transformed with the amplified Δnup97::*ura4* fragment and Ura⁺ Leu⁺ transformants were selected and confirmed for the of *nup97* null allele by PCR.

Construction of plasmids

cDNA clone of *nup97* was made by RT-PCR from total RNA obtained from wild-type *S. pombe* cells. The entire ORF of *nup97* was amplified from the genomic clone by PCR and inserted into pREP41X vectors (Maundrell, 1993).

In situ hybridization

In situ hybridization was performed as previously described (Yoon, 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)
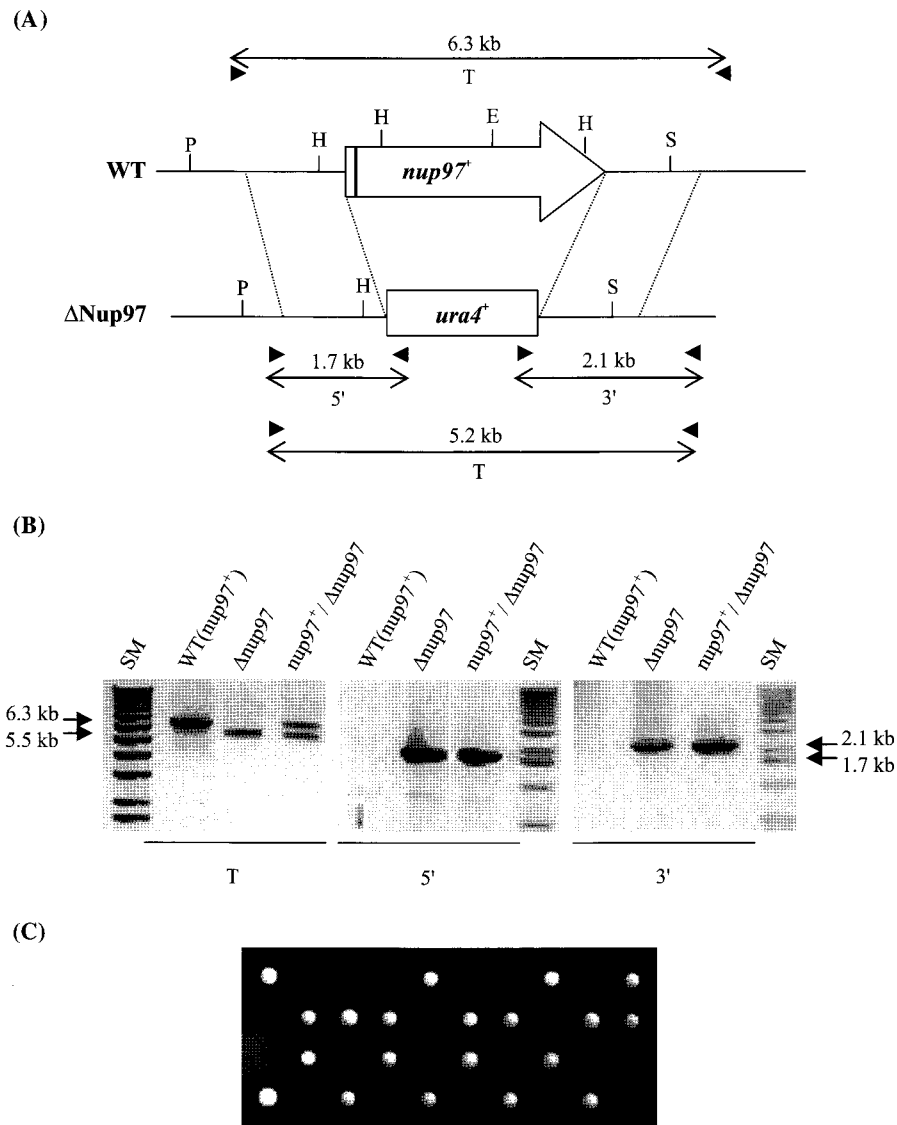
(A)

(B)

(C)

Fig. 2. The *nup97* gene is essential for vegetative growth. (A) A schematic diagram representing the constucts of the *nup97* knockout allele in *S. pombe*. Most of the *nup97* open reading frame region was substituted with the marker gene, *ura4*$^+$. The positons of PCR primers for confirmation are indicated by arrowheads and predicted sizes of PCR-amplfied fragments shown between arrowheads. E, *Eco*RI; H, *Hind*III; P, *Pst*I; S, *Sal*I. (B) Confirmation of the disruption of the *nup97* locus. PCR was performed with primers denoted in (A), using genomic DNAs isolated from wild type (WT), Diploid with a disruption in one of the *nup97* loci (*nup97*$^+$/Δ*nup97*), and Δnup97 haploid with pREP41X-Nup97 (Δ*nup97*). SM represents DNA size marker (1 kb ladder). (C) Tetrad analysis. Diploid cells with a disruption in one of the *nup97* loci were sporulated on ME plates. 10 tetrads were dissected on YES plates and were incubated for 3 days at 28°C.

was used for detecting the hybridization probe by fluorescence microscopy. 4', 6-Diamidino-2'-phenyindole (DAPI) was used for observing DNA.

Results and Discussion

Cloning of synthetic lethal gene in SLMex3

Mex67/ TAP (in yeast and metazoan, respectively) functions as the export receptor for mRNA export (Reed and Hurt, 2002). We have previously isolated three mutations that are synthetically lethal when combined with *mex67* knockout allele in *S. pombe*, to identify genes involved in mRNA export (Yoon, 2003). Synthetic lethality is described as the combining

of a mutation in a single gene with a mutation in another related gene, which leads cell to death, although mutation in each single gene separately does not cause cell death. These synthetic lethal mutants, SLMex1, SLMex2, and SLMex3, carrying knockout mutation in *mex67* and a synthetic lethal mutation in related gene, are kept viable in the absence of thiamine (-B1), owing to the expression of the *mex67* gene from the plasmid, pREP81X-Mex67, under the control of a weak thiamine-repressible *nmt1* promoter (Basi *et al.*, 1993). The repression of *mex67* expression by the addition of thiamine (+B1) results in a growth defect that is accompanied by poly(A)$^+$ RNA accumulation in the nucleus (Yoon, 2003). The corresponding synthetic lethal gene for SLMex1 was

identified as *rsm1* (Yoon, 2004).

For this study, we isolated a genomic clone, 9N2, corresponding to the *slmex3* that complement the growth defect of SLMex3 (*slmex3* Δ*mex67*/pREP81X-Mex67) cells under synthetic lethal condition (in the presence of thiamine), as mentioned in Materials and Methods. This genomic clone, 9N2 was sequenced at both ends and the DNA sequence obtained was used to search the *S. pombe* genome database (Sanger Center, UK). The *S. pombe* genome database and restriction enzyme digestion of 9N2 revealed that this genomic clone contained four open reading frames (ORFs) found in the cosmid SPCC1620 (chromosome III). To investigate which ORF is capable of complementing SLMex3, three subclones of 9N2 were constructed into pDW232 plasmid and transformed into SLMex3. As shown in Fig. 1, 9N2-1 plasmid containing SPCC1620.11 ORF was able to functionally complement the growth defect of SLMex3. Whereas, 9N2-2 and 9N2-3 carrying the other three ORFs could not restore the growth defect of SLMex3.

The SPCC1620.11 gene contains one intron and encodes an 851 amino-acid protein with predicted molecular weight of 97.5 kDa. We confirmed this intron by sequencing the cDNA obtained from total RNA. A Blast search of protein data base showed that the protein had significant homology with nucleoporins, such as Nic96p in *S. cerevisiae*, Npp106p in *S. pombe*, Nup93p in vertebrate. Alignment of amino-acid sequences in these proteins revealed that the similarity is not restricted in certain region but extends throughout the entire sequences. Accordingly, we will refer to this *S. pombe* gene as *nup97*.

NIC96 in *S. cerevisiae* encodes an abundant, essential nucleoporin (Grandi *et al.*, 1993) and is involved in protein import and nuclear pore formation (Grandi *et al.*, 1995;

Zabel *et al.*, 1996). On the other hand, *S. pombe* homologue of *NIC96*, the *npp106* gene encodes a nonessential nucleoporin involved in mRNA export (Yoon *et al.*, 1997). The *npp106-1* mutant allele was identified originally from synthetic lethal screen with *rae1-167* mutation and the deletion mutation of *npp106* (Δ*npp106*) is also synthetic lethal with *rae1-167* or Δ*mex67* (Yoon *et al.*, 1997, 2000). Although both *npp106* and *nup97* are homologous to each other and mutations in *npp106* or *nup97* are synthetic lethal with Δ*mex67*, *npp106* could not complement the growth defect of SLMex3 (data not shown), suggesting some functional divergence of these proteins.

The *nup97* gene is essential for growth

In order to determine the phenotype of the *nup97* knockout, a null mutant in a stable h^+/h^+ diploid strain was constructed by replacing the *nup97*-coding region with an *ura4*$^+$ gene using a one-step gene disruption method (Fig. 2A). The stable Ura$^+$ transformants were screened by PCR for the replacement of one of the *nup97* loci by Δ*nup97::ura4*$^+$ in diploid cells (Fig. 2B). The heterozygous diploids were allowed to sporulate, and ten tetrads were dissected. Tetrad analysis gave 2:2 segregation for viability (Fig. 2C), the two growing haploid progeny being always ura$^-$ (data not shown). Microscopic inspection of haploid cells bearing Δ*nup97::ura4*$^+$ revealed that the spores did not germinate, or germinated but stopped growth at very elongated abnormal 2-4 cells. This result indicated that the Δ*nup97::ura4*$^+$ allele is lethal to cells.

Repression of *nup97* induced defects of growth and mRNA export

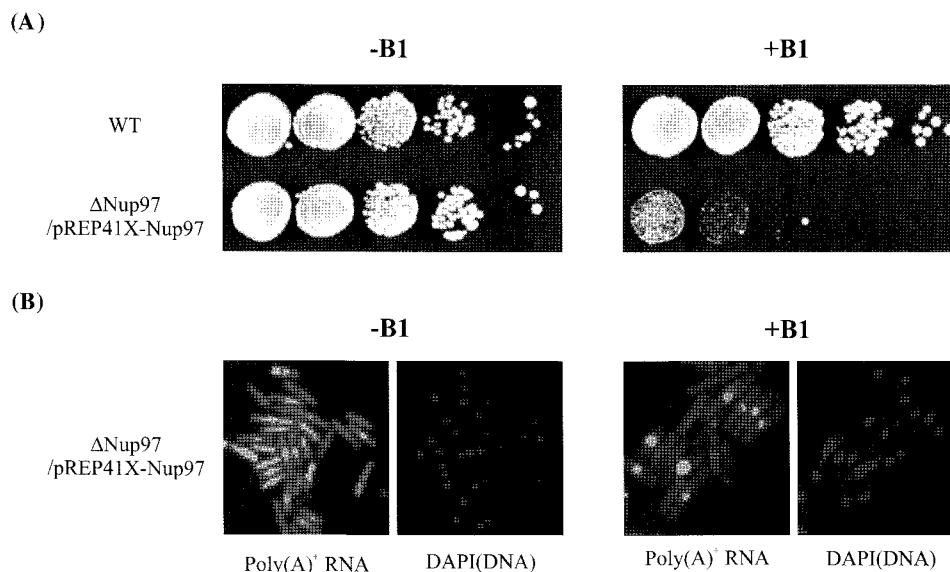Because *nup97* gene is essential for vegetative growth, we

Fig. 3. mRNA export defects of the Δ*nup97* mutants. (A) Growth of wild type (AY217) and Δnup97/pREP41X-Nup97 strains was monitored by spot assay for 5 days with (−B1) and without (+B1) expression of *nup97*. (B) Poly(A)$^+$ RNA localization in the Δ*nup97* mutants. Cells were grown to the mid-log phase in appropriately supplemented EMM medium in the absence of thiamine (−B1) at 28°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.

used a haploid strain AY217 transformed with pREP41X-Nup97 plasmid, in which *nup97* expression is under the control of a weak *nmt1* promoter and *LEU2* is used as selectable marker. This strain was transformed with the amplified Δ*nup97::ura4*$^+$ fragment and Ura$^+$ Leu$^+$ transformants were selected and confirmed for the genomic *nup97* knockout allele by PCR (Fig. 2B). In this strain, Δnup97/pREP41X-Nup97, *nup97* is expressed only from the weak thiamine-repressible *nmt* promoter on the pREP41X vector. As shown in Fig. 3A, therefore, this strain grew well in the absence of thiamine (-B1), comparable to wild type. However, repression of *nup97* expression by the addition of thiamine revealed growth defect and cells became abnormal. This result confirmed again that *nup97* is essential for vegetative growth.

To determine whether the *nup97* gene is involved in mRNA export, poly(A)$^+$ RNA distribution was examined in this strain grown under permissive (-B1) and restrictive conditions (+B1). The poly(A)$^+$ RNA in the wild type strain is distributed throughout the whole cell (Yoon *et al.*, 2000). In the case of Δ*nup97* mutants with pREP41X-Nup97, poly(A)$^+$ RNA was accumulated slightly in the nucleus even under permissive condition (Fig. 3B). However, after repression of *nup97* for 18 h in the presence of thiamine, most cells showed extensive poly(A)$^+$ RNA accumulation in the nucleus and a decrease of poly(A)$^+$ RNA in the cytoplasm (Fig. 3B). These results suggested that *nup97* affects the export of poly(A)$^+$ RNA from the nucleus.

In summary, we have isolated the *nup97* gene that encodes a nucleoporin homologous to *npp106* in *S. pombe*. Repression of *nup97* cause poly(A)$^+$ RNA accumulation in nucleus and *nup97*is genetically linked to *mex67*, suggesting that this is involved in mRNA export as like *npp106*. Both *nup97* and *npp106* are homologous to *NIC96* in *S. cerevisiae*. Nic96p is symmetrically distributed at cytoplasmic and nuclear face of the NPC, and Nic96p containing only α-solenoid fold may form the structural scaffold that provides framework for assembling the NPC (Devos *et al.*, 2006; Tran and Wente, 2006). Nic96p may have multiple roles because they are present in more than one subcomplex, the Nic96 subcomplex (composed of Nic96p, Nup152p, Nup188p, Nup170p, Nup157p, Nup59p, and Nup53p) and the Nsp1 subcomplex (comprised of Nsp1p, Nup49p, and Nup57p) (Grandi *et al.*, 1995; Zabel *et al.*, 1996; Fahrenkrog *et al.*, 1998). In *S. pombe*, it is possible that two *NIC96* homologues, *nup97* and *nup106*, may also form the structural scaffold of the NPC, but may localize in distinct subcomplexes.

Because the NPC is dynamic and NPC proteins are thought to play active roles in mRNA export, to Fig. out the functions of *nup97* and *npp106* in mRNA export would be interesting and important for the advancement of our knowledge about translocation of mRNP through NPC.

Acknowlegement

References

Alfa, C., P. Fantes, J. Hyams, M. Mcleod, and E. Warbrick. 1993.

Experiments with fission yeast. Cold Spring Harbor, Cold Spring Harbor Laboratory, NY, USA.

Barbet, N., W.J. Muriel, and A.M. Carr. 1992. Versatile shuttle vectors and genomic libraries for use with *Schizosaccharomyces pombe*. *Gene* 114, 59-66.

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.

Braun, I.C., A. Herold, M. Rode, and E. Izaurralde. 2002. Nuclear export of mRNA by TAP/NXF1 requires two nucleoporin-binding sites but not p15. *Mol. Cell. Biol.* 22, 5405-5418.

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.

Cronshaw, J.M., A.N. Krutchinsky, W. Zhang, B.T. Chait, and M.J. Matunis. 2002. Proteomic analysis of the mammalian nuclear pore complex. *J. Cell Biol.* 158, 915-927.

Devos, D., S. Dokudovskaya, F. Alber, R. Williams, B.T. Chait, A. Sali, and M.P. Rout. 2004. Components of coated vesicles and nuclear pore complexes share a common molecular architecture. *PLoS Biol.* 2, e380.

Devos, D., S. Dokudovskaya, R. Williams, F. Alber, N. Eswar, B.T. Chait, M.P. Rout, and A. Sali. 2006. Simple fold composition and modular architecture of the nuclear pore complex. *Proc. Natl. Acad. Sci. USA* 103, 2172-2177.

Erkmann, J.A. and U. Kutay. 2004. Nuclear export of mRNA: from the site of transcription to the cytoplasm. *Exp. Cell Res.* 296, 12-20.

Fahrenkrog, B., E.C. Hurt, U. Aebi, and N. Pante. 1998. Molecular architecture of the yeast nuclear pore complex: localization of Nsp1p subcomplexes. *J. Cell Biol.* 143, 577-588.

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

Grandi, P., V. Doye, and E.C. Hurt. 1993. Purification of NSP1 reveals complex formation with 'GLFG' nucleoporins and a novel nuclear pore protein NIC96. *EMBO J.* 12, 3061-3071.

Grandi, P., N. Schlaich, H. Tekotte, and E.C. Hurt. 1995. Functional interaction of Nic96p with a core nucleoporin complex consisting of Nsp1p, Nup49p and a novel protein Nup57p. *EMBO J.* 14, 76-87.

Gwizdek, C., N. Iglesias, M.S. Rodriguez, B. Ossareh-Nazari, M. Hobeika, G. Divita, F. Stutz, and C. Dargemont. 2006. Ubiquitin-associated domain of Mex67 synchronizes recruitment of the mRNA export machinery with transcription. *Proc. Natl. Acad. Sci. USA* 103, 16376-16381.

Huang, Y. and J.A. Steitz. 2005. SRprises along a messenger's journey. *Mol. Cell.* 17, 613-615.

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.

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

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

Moore, M.J. 2005. From birth to death: the complex lives of eukaryotic mRNAs. *Science* 309, 1514-1518.

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

Reed, R. and E. Hurt. 2002. A conserved mRNA export machi-

nery coupled to pre-mRNA splicing. *Cell* 108, 523-531.

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

Rout, M.P. and J.D. Aitchison. 2001. The nuclear pore complex as a transport machine. *J. Biol. Chem.* 276, 16593-16596.

Rout, M.P., J.D. Aitchison, A. Suprapto, K. Hjertaas, Y. Zhao, and B.T. Chait. 2000. The yeast nuclear pore complex: composition, architecture, and transport mechanism. *J. Cell Biol.* 148, 635-651.

Segref, A., K. Sharma, V. Doye, A. Hellwig, J. Huber, R. Luhrmann, and E. Hurt. 1997. Mex67p, a novel factor for nuclear mRNA export, binds to both poly(A)$^+$ RNA and nuclear pores. *EMBO J.* 16, 3256-3271.

Stoffler, D., B. Fahrenkrog, and U. Aebi. 1999. The nuclear pore complex: from molecular architecture to functional dynamics. *Curr. Opin. Cell Biol.* 11, 391-401.

Suntharalingam, M. and S.R. Wente. 2003. Peering through the pore: nuclear pore complex structure, assembly, and function. *Dev. Cell* 4, 775-789.

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.

Vasu, S.K. and D.J. Forbes. 2001. Nuclear pores and nuclear assembly. *Curr. Opin. Cell Biol.* 13, 363-375.

Whalen, W.A., J.H. Yoon, R. Shen, and R. Dhar. 1999. Regulation of mRNA export by nutritional status in fission yeast. *Genetics* 152, 827-838.

Yoon, J.H. 2003. Synthetic lethal mutations with *spmex67* of *Schizosaccharomyces pombe* in the mediation of mRNA export. *J. Microbiol.* 41, 115-120.

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.

Yu, J.H., Z. Hamari, K.H. Han, J.A. Seo, Y. Reyes-Dominguez, and C. Scazzocchio. 2004. Double-joint PCR: a PCR-based molecular tool for gene manipulations in filamentous fungi. *Fungal Genet. Biol.* 41, 973-981.

Zabel, U., V. Doye, H. Tekotte, R. Wepf, P. Grandi, and E.C. Hurt. 1996. Nic96p is required for nuclear pore formation and functionally interacts with a novel nucleoporin, Nup188p. *J. Cell Biol.* 133, 1141-1152.