The Journal of Microbiology, August 2008, p. 422-428 Vol. 46, No. 4
DOI 10.1007/s12275-008-0177-0

The Fission Yeast Homologue of Gle1 is Essential for Growth and Involved in mRNA Export

DongGeRaMi Moon[1], Jin-Ah Bae[2], Hyun Jin Cho[1], and Jin Ho Yoon[1]*

[1]Department of Biology, Institute of Basic Sciences, and [2]Department of Education, Sungshin Women's University, Seoul 136-742, Republic of Korea

(Received July 14, 2008 / Accepted July 22, 2008)

We have isolated Gle1 homologue (named as *spgle1*) as a partial multicopy suppressor of the synthetic lethality of *rae1-167 elf1-21* in fission yeast *Schizosaccharomyces pombe*. The *spgle1* is also able to complement partially temperature-sensitive phenotype of *rae1-167* only at a lower restrictive temperature. The *spgle1* gene contains one intron and encodes a 480 amino-acid protein with predicted molecular weight of 56.2 kDa. We showed that *spgle1* gene is essential for vegetative growth and functional Gle1-GFP protein is localized mainly in NPC. The accumulation of poly(A)$^+$ RNA in the nucleus is exhibited when expression of *spgle1* is repressed or over-expressed. These results suggest that the spGle1 protein is also involved in mRNA export in fission yeast.

***Keywords*:** mRNA export, *Schizosaccharomyces pombe*, *spgle1*

In eukaryotes, nuclear export of mRNA to cytoplasm takes place through the nuclear pore complex (NPC) embedded in the nuclear envelope. mRNA export is a evolutionally conserved complex process, which is linked to the other steps of mRNA metabolism and surveillance (Reed and Hurt, 2002; Stutz and Izaurralde, 2003; Erkmann and Kutay, 2004). Several soluble mRNA export factors as well as specific nucleoporins (proteins composing of NPC) are involved in mRNA export (Tran and Wente, 2006; Köhler and Hurt, 2007). One of conserved key export factors is a heterodimeric mRNA carrier, Mex67p-Mtr2p in yeast and TAP/NXF-p15/NXT in metazoan (Segref *et al*., 1997; Grüter *et al*., 1998). These mRNA carriers function not only by linking mRNPs complex to NPCs and transporting mRNPs through the NPCs, but also by connecting transcription and pre-mRNA processing steps to mRNA export (Rodriguez *et al*., 2004).

Other essential mRNA export factors are NPC-associated proteins, such as Dbp5, Gle1, and Rae1/Gle2 (Brown *et al*., 1995; Del Priore *et al*., 1996; Murphy and Wente, 1996; Snay-Hodge *et al*., 1998; Tseng *et al*., 1998). All of these proteins are interacted genetically and physically with Mex67p/TAP. The DEAD-box protein Dbp5p has ATPase activity, which is activated on the cypoplasmic face of NPC by the Gle1p and further stimulated by the small molecule, inositol hexakiphosphate (IP_6) (Alcázar-Román *et al*., 2006; Weirich *et al*., 2006). This activity is required to dissociate Mex67p and Nab2p from exported mRNP by triggering specific RNA: protein remodeling events (Lund and Guthrie, 2005; Tran *et al*., 2007). Rae1/Gle2 forms a complex with nucleoporin Nup98p, binds mRNA, and interacts with other mRNA export factors to mediate mRNA export (Pritchard *et al*., 1999; Yoon *et al*., 2000; Blevins *et al*., 2003). However, how the actions of Rae1/Gle2 are coordinated with the other factors for mRNA export is largely unknown.

In *Schizosaccharomyces pombe*, *rae1* is essential for growth and mRNA export, whereas *spmex67* (*S. pombe mex67*) is not (Brown *et al*., 1995; Yoon *et al*., 2000). The temperature-sensitive *rae1-167* mutant shows rapid accumulation of mRNA in the nucleus and a cell cycle arrest at the G_2/M boundary at restrictive temperature above 30°C (Brown *et al*., 1995; Yoon *et al*., 1997). Three synthetic lethal mutants were screened by random mutation using the *rae1-167* mutant cells to identify the genes related to *rae1* for mRNA export (Yoon *et al*., 1997). Synthetic lethality is described as the combination of a mutation in a single gene with a mutation in another related gene (two mutant genes in one cell), which leads cell to death, although mutation in each single gene separately (only one mutant gene in a cell) does not cause cell death. These mutant cells harboring mutated gene that is synthetic lethal with *rae1-167* allele, is kept viable in the absence of thiamine (-B1), owing to the expression of the *rae1* gene from the plasmid, pREP81X- Rae1, under the control of a thiamine-repressible *nmt1* promoter (Basi *et al*., 1993). One of synthetic lethal genes was identified as *elf1* that encodes a member of the ABC class of ATPase (Kozak *et al*., 2002).

In this study, we described the isolation of a fission yeast homologue of *Gle1* (*spgle1*) by partial multicopy suppressor of the growth defect in the *rae1-167 elf1-21* synthetic lethal mutants, SL21. In addition, the repression or over-expression of *spgle1* was shown to cause defects in both growth and mRNA export. These results suggested that *spgle1* is also involved in the mRNA export in *S. pombe*.

* To whom correspondence should be addressed.
(Tel) 82-2-920-7675; (Fax) 82-2-920-7675
(E-mail) jhoyoon@sungshin.ac.kr

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)
SL21	*h⁻ leu-32 ura4-D18 elf1-21 rae1-167*/ pREP81X-Rae1	Yoon *et al.* (1997)
AY208	*h⁻ leu-32 ura4-D18 rae1-167*	Yoon *et al.* (1997)
AY217(3X-Gle1)	*h⁻ leu-32 ura4-D18*/ pREP3X-Gle1	This study
ΔspGle1(81X-Gle1)	*h⁻ leu-32 ura4-D18 spgle1::ura4*/ pREP81X-Gle1	This study
SP286(Δ*gle1*)	*h⁺/h⁺ leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-210/ade6-216 spgle1/spgle1::ura4*	This study

Materials and Methods

Strains and culture

The *S. pombe* strains used in this study are listed in Table 1. The basic genetic and cell culture techniques used 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 pREP series 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). *Escherichia coli* TOP10 strain was used for plasmid propagation and selection.

Isolation of multicopy suppressor gene for SL21

SL21 cells were transformed with a partial *Sau*3A genomic library that was cloned into the *Sal*I site of pUR18 (Barbet, 1992). Transformants were isolated, which could grow in the presence of thiamine at 28°C. Plasmids isolated from these transformants were enriched through *E. coli* transformation and re-transformed into SL21 for confirmation. A plasmid that rescued partially synthetic lethality of SL21 was sequenced at both ends of insert and the DNA sequence was used to search the *S. pombe* genome database (http://www.sanger.ac.uk/Projects/S_pombe/, Sanger Center, UK). A 5.7 kb insert of the plasmid contained three full-length open reading frames (ORFs) and each gene was subcloned into pDW232 and transformed into SL21 to investigate which one could complement the growth defect of SL21 in the presence of thiamine.

Construction of *spgle1* null mutants

The Δ*spgle1::ura4* null mutation was constructed as follows. A 1 kb DNA fragment upstream from *spgle1* ORF with *Xba*I and *Not*I site at the ends was amplified by PCR. A 0.6 kb DNA fragment downstream from *spgle1* ORF with *Not*I and *Xho*I site at the ends was also amplified by PCR. The deletion was then constructed by ligation of the 5' *Xba*I-*Not*I and the 3' *Not*I-*Xho*I fragments into a pBluescript SK(+) vector (Stratagene, USA) cut with *Xba*I and *Xho*I. A *Not*I fragment bearing *ura4* marker gene was inserted into *Not*I site at the deletion junction. This plasmid was digested with *Xba*I and *Xho*I, and the gel-purified Δ*spgle1::ura4* fragment was transformed into the SP286 diploid strain. Ura$^+$ transformants were selected and screened by PCR and Southern blotting for the disruption of one of the *spgle1* 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 *spgle1* allele (Fig. 2).

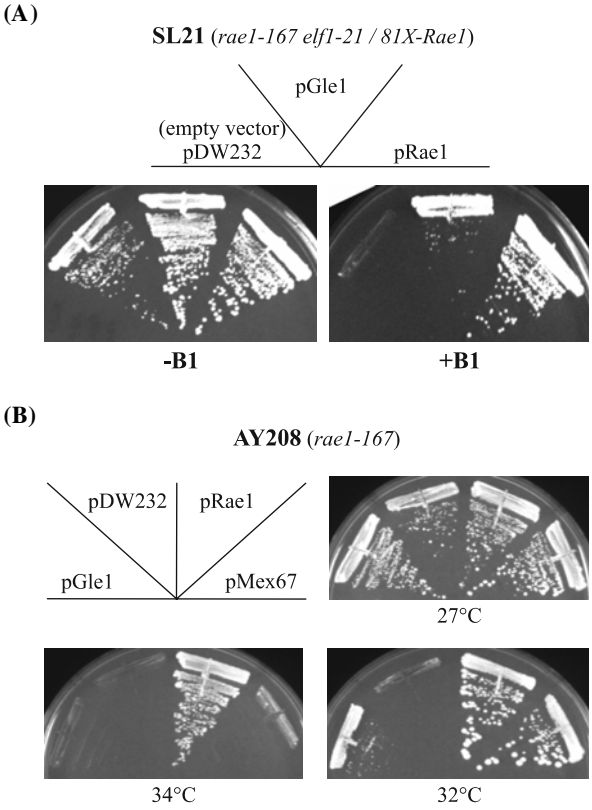

Fig. 1. (A) Partial suppression of growth defects of synthetic lethal mutant SL21 by spGle1p. SL21 cells transformed with pDW232, pGle1, and pRae1 were streaked onto EMM agar in the absence (-B1) and the presence (+B1) of thiamine. A vector plasmid without an insert, pDW232, is used as a negative control and pRae1 as positive control. *spgle1* (pGle1) and *rae1* (pRae1) gene are expressed from their own promoters carried in the pDW232 plasmid. Cells were grown for 4 days at 27°C. (B) Partial suppression of the ts phenotype of the *rae1-167* mutation by spGle1p. The mutant AY208 (*rae1-167*) cells transformed with different plasmids are represented. Cells were grown for 4 days at different temperatures as indicated under panels.

Because *spgle1* gene is essential for vegetative growth, we used a haploid strain AY217 transformed with pREP81X-Gle1 plasmid in which *spgle1* expression is under the control of *nmt* promoter. This strain was transformed with the amplified Δ*spgle1::ura4* fragment and Ura$^+$ Leu$^+$ transformants were selected and confirmed for the of *spgle1* null allele by PCR.

Construction of plasmids

cDNA clone of *spgle1* was made by RT-PCR from total RNA obtained from wild-type *S. pombe* cells and confirmed by sequencing. The cloning of entire ORF of *spgle1* into pREP series vectors (Maundrell, 1993) was conducted by first creating *Xho*I and *Eco*RV sites immediately upstream of the initiation codon and downstream of the stop codon of *spgle1*, respectively, by PCR using the cDNA clone as a temperate. The *Xho*I-*Eco*RV digested PCR product was then inserted into pREP series vectors cut with *Sal*I and *Sma*I.

In situ hybridization

In situ hybridization was performed as previously described (Yoon *et al.*, 2000). Oligo-(dT)$_{50}$ carrying an α-digoxygenin at the 3' end was used as the hybridization probe. FITC-anti-

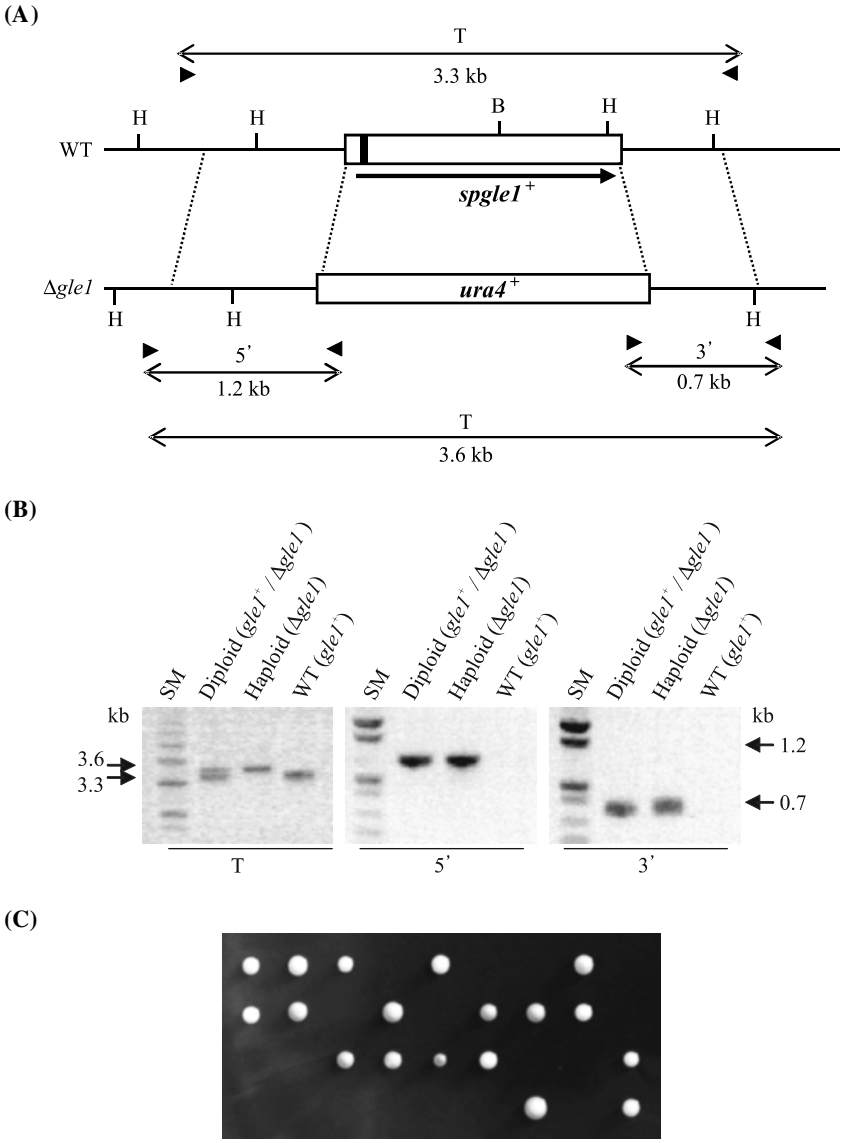

Fig. 2. The *spgle1* gene is essential for vegetative growth. (A) A schematic diagram representing the constucts of the *spgle1* knockout allele in *S. pombe*. Most of the *spgle1* ORF region was substituted with the marker gene, *ura4*$^+$. The positons of PCR primers for confirmation are indicated by arrowheads and predicted sizes of PCR-amplfied fragments shown between arrowheads. B, *Bam*HI; H, *Hin*dIII. (B) Confirmation of the disruption of the *spgle1* 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 *spgle1* loci (*gle1*$^+$/Δ*gle1*), and Δ*gle1* haploid with pREP81X-Gle1 (Δ*gle1*). SM represents DNA size marker (1 kb ladder). (C) Tetrad analysis. Diploid cells with a disruption in one of the *spgle1* loci were sporulated on ME plates. 10 tetrads were dissected on YES plates and were incubated for 4 days at 27°C.

digoxygenin Fab antibody (Roche Applied Science, Germany) was used for detecting the hybridization probe by fluorescence microscopy. 4', 6-Diamidino-2'-phenyindole (DAPI) was used for observing DNA.

Results and Discussion

Partial suppression of *rae1-167 elf1-21* synthetic lethality and the temperature-sensitive phenotype of the *rae1-167* mutation by spGle1p

The *S. pombe* SL21 is a synthetic lethal strain that was originally screened by random mutation using the temperature-sensitive (*rae1-167* allele) mutant cells (Yoon *et al*., 1997). The corresponding synthetic lethal gene for *sl21* was identified as *elf1* that encodes a member of the ABC class of ATPase (Kozak *et al*., 2002). In this study, we isolated a genomic clone as a multicopy suppressor that complement partially the growth defect of SL21 cells under synthetic lethal condition (in the presence of thiamine), as mentioned in 'Materials and Methods'. This genomic clone contained 5.7 kb insert and both ends of insert DNA was sequenced. 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 patterns of the clone revealed that this genomic clone contained three full-length ORFs found in the cosmid c31E1 (chromosome II). To investigate which ORF is capable of complementing SL21, three subclones harboring each ORF were constructed into pDW232 plasmid and transformed into SL21. Only one plasmid containing SPBC31E1.05 ORF was able to complement partially the growth defect of SL21 (Fig. 1A).

The SPBC31E1.05 gene contains one intron and encodes a 480 amino-acid protein with predicted molecular weight of 56.2 kDa and an isoelectric point (pI) of 9.7. We confirmed this intron by sequencing the cDNA obtained from total RNA (data not shown). A Blast search of protein data base showed that the protein had significant homology with mRNA export factor Gle1 (Del Priore *et al*., 1996; Murphy and Wente, 1996; Watkins *et al*., 1998). Accordingly, we will refer to this gene as *S. pombe gle1* (*spgle1*). Although *S. pombe* Gle1 (spGle1, 480 residues) is smaller than *S. cerevisiae* Gle1 (scGle1, 538 residues) and human Gle1 (hGle1A, 659 residues), alignment of amino-acid sequences in these proteins revealed that structural and sequence similarities are not restricted in certain region but extends throughout the entire sequences. Similar to scGle1p and hGle1p, the middle region (residues 110∼245) of spGle1p is highly charged and is predicted to have a very high potential to form a coiled-coil structure. Especially, the C-terminal region (residues 201∼445) of spGle1p also showed extensive similarity (around 50∼80%) to the corresponding regions of Gle1p in the other organisms. However, the leucine-rich NES (nuclear export signal) found in scGle1p, which seems to be important for mRNA export in *S. cerevisiae* (Murphy and Wente, 1996), is not conserved in hGle1p or spGle1p.

Because *spgle1* was isolated as multicopy suppressor gene

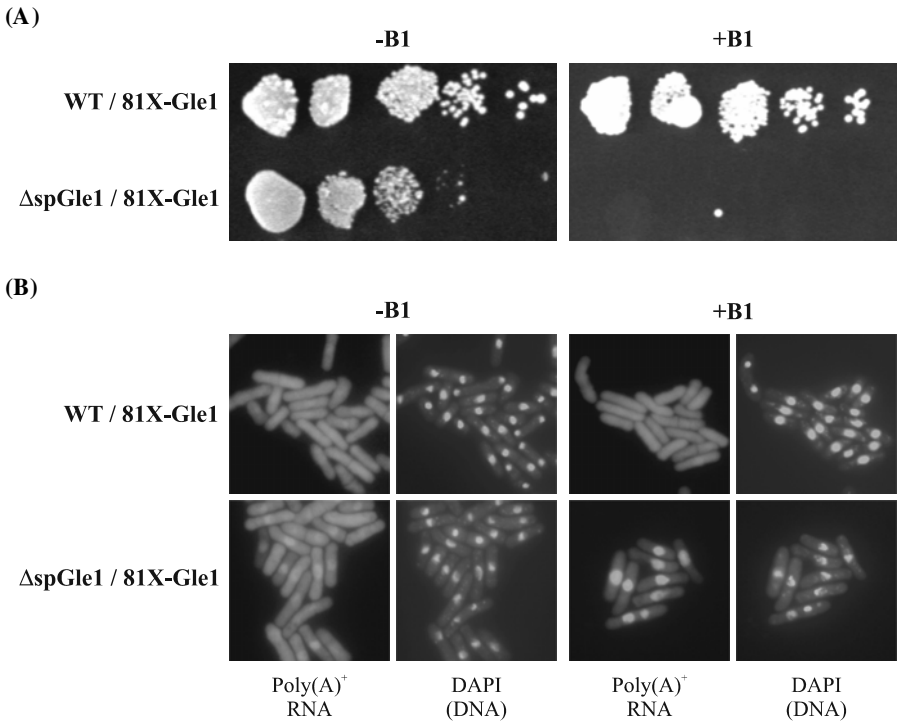

Fig. 3. Δ*spgle1* mutants showed the defects of growth and mRNA export. (A) Growth of wild type (AY217 carrying pREP81X-Gle1 plasmid) and ΔspGle1/p81X-Gle1 strains (Δ*spgle1* mutants carrying pREP81X-Gle1 plasmid) was monitored by spot assay for 5 days at 27°C with (+B1) and without (-B1) expression of *spgle1*. (B) Poly(A)$^+$ RNA localization in the Δ *spgle1* mutants. Cells were grown to the mid-log phase in appropriately supplemented EMM medium in the absence of thiamine (-B1) at 27°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.

of *rae1-167 elf1-21* synthetic lethality, we wanted to check whether *spgle1* is also able to suppress the temperature-sensitive (ts) phenotype of the *rae1-167* mutation. As shown in Fig. 1B, pGle1 plasmid harboring *spgle1* gene is able to suppress partially the ts phenotype of *rae1-167* mutation at the only lower restrictive temperature of 32°C, compared to *spmex67* that is known to extragenic suppressor of the *rae1-167* mutation (Yoon *et al*., 2000). However, both *spgle1* and *spmex67* could not suppress the ts phenotype of *rae1-167* mutation at restrictive temperature above 34°C.

The *spgle1* gene is essential for growth

In order to determine the phenotype of the *spgle1* knockout, a null mutant in a stable h^+/h^+ diploid strain was constructed by replacing the *spgle1*-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 selection of diploid cells, in which one of two *spgle1* loci was substituted with *ura4* (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*spgle1::ura4* revealed that the spores did not germinate, or germinated but stopped growth at elongated abnormal cells. This result indicated that the Δ*spgle1::ura4* allele is lethal to cells.

Repression or over-expression of *spgle1* inhibits growth and mRNA export

Because *spgle1* gene is essential for vegetative growth, we decided to construct a strain, in which *spgle1* expression can be repressed. For this, we used a haploid strain AY217 carrying 81X-Gle1 plasmid, in which *spgle1* expression is under the control of a weak *nmt1* promoter on pREP81X vector (*LEU2* used as selectable marker). This strain was transformed with the amplified Δ*spgle1::ura4* fragment for replacing the genomic *spgle1*-coding region with an *ura4* gene. Ura^+ Leu^+ transformants were selected and the genomic *spgle1* knockout allele was confirmed by PCR (Fig. 2B). In this strain, ΔspGle1/81X-Gle1, *spgle1* is expressed only from the weak thiamine-repressible *nmt1* promoter on the 81X-Gle1 vectors. As shown in Fig. 3A, therefore, this strain grew in the absence of thiamine (-B1), although its growth is slower than that of wild type cells. This suggested that expression of *spgle1* from the weak *nmt1* promoter may be not enough to retain the normal growth rate. However, repression of *spgle1* expression by the addition of thiamine (+B1) caused the immediate cessation of cell growth. These results confirmed again that *spgle1* is essential for vegetative growth.

To determine whether the *spgle1* 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 (Fig. 3B). In the case of *spgle1* mutants with 81X-Gle1, poly(A)$^+$ RNA was accumulated slightly in the nucleus even under permissive condition, probably because of low level of *spgle1* expression as mentioned above (Fig. 3B). However, after repression of *spgle1* 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 *spgle1* affects the export of

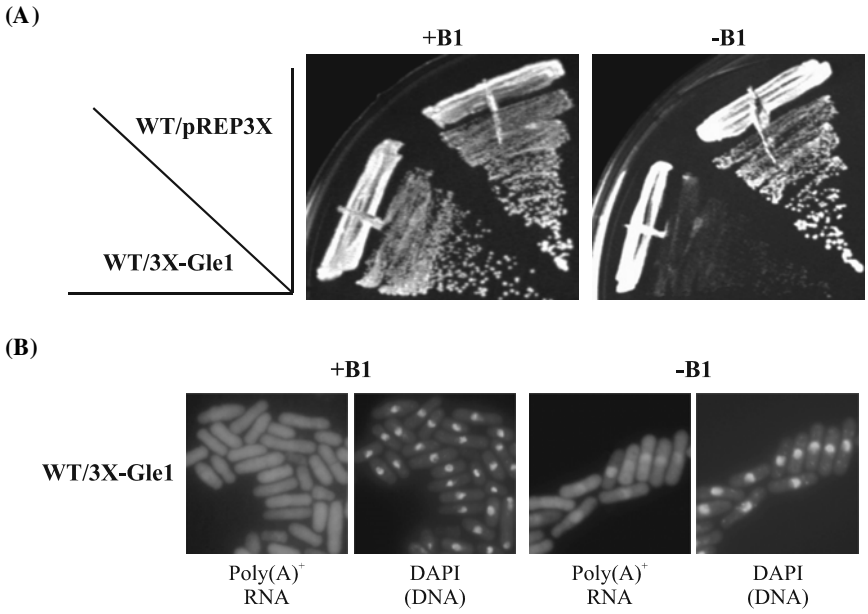

Fig. 4. (A) Overexpression of *spgle1* result in growth retardation. Haploid wild-type (AY217) cells carrying pREP3X plasmid without a insert (WT/pREP3X) or pREP3X-Gle1 (WT/3X-Gle1) were streaked onto EMM agar in the absence (-B1) and the presence (+B1) of thiamine. Cells were incubated for 4 days at 27°C. (B) Overexpression of *spgle1* showed slight defects in mRNA export. Cells were grown to the mid-log phase in appropriately supplemented EMM medium in the presence of thiamine (+B1) at 27°C. The cells were then washed and shifted to EMM medium without thiamine (-B1) and were grown for 18 h. Coincident DAPI staining is shown in the right panels.

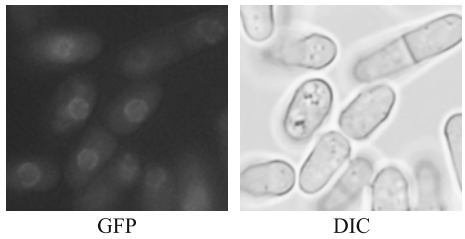

Fig. 5. Localization of spGle1p fused to GFP. Cells were grown to the mid-log phase in appropriately supplemented EMM medium in the presence of thiamine (+B1) at 27°C. Coincident differntial interference contrast (DIC) images are also shown in the right panels.

poly(A)$^+$ RNA from the nucleus to the cytoplasm.

On the other hand, we want to check whether over-expression of *spgle1* also affects the growth and mRNA export. For this, we used a much stronger *nmt1* promoter in the pREP3X vector than that in pREP81X (Forsberg, 1993). When *spgle1* was over-expressed from a stronger promoter on 3X-Gle1 plasmid in addition to expression from genomic *spgle1* locus, we tested if cell growth and nuclear export of poly(A)$^+$ RNA is inhibited in the wild-type cells. As shown in Fig. 4, over-expression of *spgle1* in the absence of thiamine (-B1) cause retardation of growth and this inhibition was accompanied by slight accumulation of poly(A)$^+$ RNA in nucleus (Fig. 4). However, when *spgle1* expression from plasmid was repressed in the presence of thiamine (+B1) as a negative control, cell growth and the distribution of poly(A)$^+$ RNA looked normal as like wild-type cells. These results suggest that over-expressed spGle1p likely interact with and titrate out other proteins that are essential for growth and nuclear export of RNA.

spGle1p fusion protein predominantly localized in the nucleus pheriphery

To analyze spGle1p further, the subcellular localization of spGle1p tagged with GFP (green fluorescent protein) was determined. This fusion protein was functional since it complemented the growth defect of ΔspGle1 cells. When observed with fluorescence microscope, Gle1-GFP signal was detected in cytoplasm and nucleus, but predominantly concentrated at the nuclear periphery in a punctuate pattern, which was typical of yeast NPC. This localization pattern of spGle1p is similar to that of scGle1p and hGle1p, suggesting that spGle1p also facilitates mRNA export at NPC with factors similar to other organisms.

Acknowlegements

This work was supported by the Sungshin Women's University Research Grant of 2005.

References

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.

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.

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.

Blevins, M.B., A.M. Smith, E.M. Phillips, and M.A. Powers. 2003. Complex formation among the RNA export proteins Nup98, Rae1/Gle2, and TAP. *J. Biol. Chem.* 278, 20979-20988.

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.

Del Priore, V., C.A. Snay, A. Bahr, and C.N. Cole. 1996. The product of the *Saccharomyces cerevisiae* RSS1 gene, identified as a high-copy suppressor of the rat7-1 temperature-sensitive allele of the RAT7/NUP159 nucleoporin, is required for efficient mRNA export. *Mol. Biol. Cell* 7, 1601-1621.

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.

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

Grüter, P., C. Tabernero, C. Von Kobbe, C. Schmitt, C. Saavedra, A. Bachi, M. Wilm, B.K. Felber, and E. Izaurralde. 1998. TAP, the human homolog of Mex67p, mediates CTE-dependent RNA export from the nucleus. *Mol. Cell* 1, 649-659.

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.

Lund, M.K. and C. Guthrie. 2005. The DEAD-box protein Dbp5p is required to dissociate Mex67p from exported mRNPs at the nuclear rim. *Mol. Cell* 20, 645-651.

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.

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

Murphy, R. and S.R. Wente. 1996. An RNA-export mediator with an essential nuclear export signal. *Nature* 383, 357-360.

Pritchard, C.E., M. Fornerod, L.H. Kasper, and J.M. Van Deursen. 1999. RAE1 is a shuttling mRNA export factor that binds to a GLEBS-like NUP98 motif at the nuclear pore complex through multiple domains. *J. Cell Biol*. 145, 237-254.

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

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.

Snay-Hodge, C.A., H.V. Colot, A.L. Goldstein, and C.N. Cole. 1998. Dbp5p/Rat8p is a yeast nuclear pore-associated DEAD-box protein essential for RNA export. *EMBO J.* 17, 2663-2676.

Stutz, F. and E. Izaurralde. 2003. The interplay of nuclear mRNP assembly, mRNA surveillance and export. *Trends Cell Biol*. 13,

319-327.

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

Tran, E.J., Y. Zhou, A.H. Corbett, and S.R. Wente. 2007. The DEAD-box protein Dbp5 controls mRNA export by triggering specific RNA:protein remodeling events. *Mol. Cell* 28, 850-859.

Tseng, S.S., P.L. Weaver, Y. Liu, M. Hitomi, A.M. Tartakoff, and T.H. Chang. 1998. Dbp5p, a cytosolic RNA helicase, is required for poly(A)$^+$ RNA export. *EMBO J.* 17, 2651-2662.

Watkins, J.L., R. Murphy, J.L. Emtage, and S.R. Wente. 1998. The human homologue of *Saccharomyces cerevisiae* Gle1p is required for poly(A)+ RNA export. *Proc. Natl. Acad. Sci. USA* 95, 6779-6784.

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., 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.