윤 진 호 교수지도

석사학위 청구논문

The Analysis of SUMOylated Nup184 involved in mRNA export

2012

성신여자대학교 대학원

생 물 학 과

채 애 리

The Analysis of SUMOylated Nup184 involved in mRNA export

윤 진 호 교수지도

이 논문을 석사학위논문으로 제출함

2011년 11월

성신여자대학교 대학원

생 물 학 과

채 애 리

인 준 서

채애리의 석사학위 논문으로 인준함.

심사위원 _____(인)

심사위원 _____(인)

심사위원 _____(인)

성신여자대학교 대학원

Abstract

The Analysis of SUMOylated Nup184 involved in mRNA export

Airee Chae
Department of Biology
Graduate School
Sungshin Women's University

The *nup184* gene in fission yeast *Schizosaccharomyces pombe* is a nucleoporin which is component of nuclear pore complexes (NPC) that are massive protein assemblies, perforating the nuclear envelope (NE). Even though the *nup184* is not essential for growth, Δ*nup184* null mutant shows retardations of both growth and mRNA export in nutrient-rich medium (YES). Nup184 protein has seven supposed SUMO consensus sequences known as the only spot where SUMO can conjugate as follows; 728LKTD, 1055VKLD, 1091FKHE, 1212VKED, and 1554LKIE. Therefore, it is thought of as being modified by SUMO. SUMO protein is composed of 97 amino-acid and similar to ubiquitin. That is encoded by *pmt3* and *smt3* in *S. pombe* and *S. cerevisiae*, respectively. SUMO is post-translationally attached to and detached from one or more lysine residues on target protein to modify their function. SUMO modification (SUMOylation) has a variety of cellular functions, including roles in transcription, DNA damage responses, the cell cycle, nuclear transport, and so on. Here we investigate whether Nup184 protein is

SUMOylated or not, and if so, what the function of SUMO modification of Nup184p is and which region is required for SUMOylation and growth complementation. We showed that high molecular weight forms of Nup184p which is SUMO-modified using immunoprecipitation and Western blotting. We constructed serially truncated Nup184 mutants to check which could complement the growth retardation and defects of mRNA export in nutrient-rich medium (YES). And we also make the Nup184 constructs that have point mutated sequence at lysine in the middle of SUMO consensus sequence, which region is required for SUMO modification. Among these constructs, C-terminal deleted Nup184 was severely SUMOylated as compared with the others and was not complement on complete medium. Moreover, localization of poly(A)$^+$ RNA in these constructs was accumulated in their nucleus. This result proposed that C-terminal end of the Nup184 might play a important role in controlling SUMO modification negatively, gene complementation, and poly(A)$^+$ RNA exporting.

Contents

List of Tables

List of Figures

I. Introduction

The compartmentalization of genetic materials inside the cell's nucleus is the significant feature of eukaryotes unprecedented in prokaryotes. Separation of transcription and translation of eukaryotic cells has performed by the physical boundary of the nuclear envelope (NE). Nuclear envelope which has the outer nuclear membrane and the inner nuclear membrane has allowed partition both spatially and temporally to accomplish intricate regulation more than prokaryotes (Gandhi *et al*., 2010).

Nuclear pore complexes (NPCs) are massive protein assemblies that perforate the nuclear envelope. NPCs are composed of about 30 different nuclear pore proteins called nucleoporins and they have molecular weight of 60MD in yeast. NPC is based on a doughnut-like central body, ~120nm in diameter and 70nm in width, which have eight-fold symmetric central construction called the spoke complex. The eight spoke unites encircle the nuclear pore through which active transport occurs (Murray, 2007).

Filaments extend from the central body of nuclear pores into both the cytoplasm and the nucleus. Especially, in the case of filaments toward to the nuclear, those form a nuclear basket structure blow the body of the NPC (Murray, 2007). They function as doorkeepers of the nucleus, carrying out an enormous exchange of RNA, proteins, and other factors between the nucleoplasm and the cytoplasm (Gandhi *et al.*, 2010).

The number of NPCs per cell differs greatly with cell size and activity. A mature *Xenopus* oocyte has about 60 NPCs/μm^2 yielding ~5 x 10^7 NPCs/nucleus and yeast cells have ~200 NPCs/nucleus. In yeast, the number of NPC was discovered to increase gradually in the cell cycle, starting in the G1-phase and hitting the highest point in the S-phase. NPC construction seems to occur continuously during the cell cycle (Roderick., 2008).

Molecules smaller than approximately 20~40kDa in mass are transported cross the NPC in a diffusion-controlled way. However, the transports of molecules above this mass either into or out are specifically regulated. Most cargo proteins bear either nuclear localization sequences (NLSs) or nuclear export sequences (NESs) that are specifically recognized by carrier proteins, exportins or importins, collectively referred to karyopherins (kaps) (Thomas., 2005).

Most transporting pathways utilize a homologous family of β-karyopherins. Importin-β import many proteins (often using importin-α as an adaptor) though a few proteins are imported using other importins such as transportin. Exporting of numerous proteins including snRNAs (small nuclear RNA) uses the general export receptor, CRM1 (also known as exportin-1), whereas miRNAs and tRNAs are exported by exportin-5 and exportin-t, respectively (all of which are importin-β homologues) (Murray, 2007). In some case, adaptor proteins are supposed to be significant because the interaction of carriers with mRNAs is feeble and nonspecific.

Karyopherins share an N-terminal domain involved in binding the small GTPase Ran, and probably mediate movement of their cargoes through interactions with both Ran-GTP and FG nucleoporins (Daniel *et al*., 2001). Directionality is achieved by the asymmetric distribution of Ran, which changes the conformation of key structural loops in Ran, and cycles between GTP- and GDP-bound states. Ran exists in a GTP-bound state in the nucleus and a GDP-bound state in the cytoplasm. Chemical gradient of the RanGTP-RanGDP across the nuclear envelope is made by the action of two regulators, RanGEF (Ran-GDP-exchange factor) in the nucleus and RanGAP (Ran-GTPase-activating protein) in the cytoplasm, and creates a driving force for bidirectional nucleo-cytoplasmic transport processes (Alwin *et al*., 2007). RanGTP releases cargo from importing kaps in nucleus. Exporting kaps, on the other hand, requires Ran-GTP to bind their cargo (Thomas., 2005).

The export of tRNA, miRNA, snRNA, and rRNA follows general Ran-dependent pathway as mentioned above. However, the export of general mRNA is mechanistically dissimilar as it uses a transport receptor that is unrelated to karyopherins and is not straightly dependent upon the RanGTP-RanGDP gradient (Ran-independent pathway). Furthermore, a large number of supplementary export factors assist the mRNA export with their receptor (Alwin *et al*., 2007). Specifically, Mex67-Mtr2 complex is thought of the general mRNA export receptor in yeast. The Mex67-Mtr2 complex is necessary to bind with Yra1, its adaptor protein (Mette *et al*., 2005).

Post-translational regulatory process is an effective and rapid manner of

controlling the activity of proteins. Many kinds of regulatory process have been identified and molecules related to regulatory process can be attached post-translationally to target proteins. Among them, the reversible conjugation of the SUMO which is acronym for a small ubiquitin-like modifier to protein substrates (SUMOylation) is emerging as a major post-translational modification in eukaryotes (Andrew *et al*., 2009). SUMOylation can change the function of target proteins by regulating protein-protein interactions, which can cause altered cellular functions including roles in transcription factors, cytoskeleton components, and metabolic enzymes, DNA damage responses, the cell cycle, nuclear transport, subcellular localization, and so on.

SUMO is encoded in *pmt3* and *smt3* in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, respectively. In *S. cerevisiae*, *smt3* is an essential gene; however, *pmt3* is not essential for growth in *S. pombe* (Kevin *et al*., 2010).

The *nup184* in fission yeast *Schizosaccharomyces pombe* is nucleoporin and encodes 1564 amino-acids protein which has 176.9kDa predicted molecular weight. Whalen *et al*. (1999) discussed that the *nup184* null allele is synthetically lethal with *rae1-167,* important gene related to mRNA export. Although the *nup184* is not essential for growth, Δ*nup184* null mutants show impairments of both growth and mRNA export in nutrient-rich medium (YES), suggesting nutritional status can control poly(A)$^+$ RNA export. Perhaps the predicted Nup184p is analogous to Nup188p of *Saccharomyces cerevisiae*.

Nup184 has seven supposed SUMO consensus sequences, ψKxE/D (where ψ represents large hydrophobic amino acid) known as the only spot where SUMO can conjugate.

In this study, we show whether Nup184 actually SUMOylated or not and phenotypes which were caused by various Nup184 mutations (serially truncated and point mutants).

Ⅱ. Materials & Methods

Ⅱ.1. Strains and culture conditions

Ⅱ.1.1. Strains

Table 1 gives fission yeast *Schizosaccharomyces pombe* strains used in this study including wild type AY217, SP286.

For amplification of recombinant plasmids, *E. coli* Top10' (Invitrogen, U.S.A.) is used and genotype of this strain is described in Table 1.

Ⅱ.1.2. Culture media

Cell culture techniques' and genetic treatments were based on *S. pombe* standard method (Alfa *et al*., 1993; Moreno *et al*., 1991). For vegetative growth, EMM (Edinburgh Minimal Medium), PMG (Pombe Glutamate Medium), and YES (Yeast Extract with Supplements) media were basically used. Solid media were made by adding 2% Bacto agar (BD, U.S.A.). Yeast cells incubated at 29℃ for 3 days were inoculated into liquid media and maintained with shaking at 29℃.

Appropriately supplemented EMM media were used to express genes from pREP plasmids containing *nmt*-promoter. 15μM thiamine (Sigma, U.S.A.) was added in EMM and PMG media for full repression of the *nmt*-promoter (Forsburg, 1993). To select kanamycine-resistant (kan^r) colonies, YES solid

medium with 100㎍/㎖ G418 (Duchefa, Netherlands) was used. *S. pombe* culture media used in this study are listed in Table 4.

E. coli were grown in LB (Luria-Bertani) medium at 37℃. Antibiotic selection for plasmids (pREP series, pZA69u, pDW232) was done with 100㎍/㎖ ampicillin in LB medium (LA).

Ⅱ.2. Plasmids

Serial truncated and point mutated *nup184* were constructed by PCR-mediated method based on pZA69u plasmid. For *pmt3*GG construction, pREP41X was used. The pREP41X and the pZA69u express LEU2 and *ura4*$^{+}$ as a selectable marker, respectively. All plasmids have ampicillin-resistance gene for antibiotic selection in *E. coli*.

The *nmt*-promoter, located at the 5'-end of multi-cloning site (MCS) of pREP series and pZA69u series, directs transcription of genes inserted into MCS. It is a repressible promoter and is controlled by thiamine.

Ⅱ.3. Enzymes

A variety of restriction endonucleases and T4 ligase were used for sub-cloning (NEB, U.K.). *Taq* DNA polymerase was used for amplification of specific DNA fragments (TAKARA, Japan).

Table 1. Strains used in this study

Strain	Genotype	Source
972$^-$	*h*$^-$ (wild type)	Leupold (1970)
AY217	*h*$^-$ *leu1-32 ura4-d18*	Yoon *et al.* (2000)
SP286	*h*$^+$/*h*$^+$ *leu1-32/leu-32 ura4-d18/ura4-d18 ade6-M210/ade6-M216*	Matsumoto and Beach (1991)
NU1	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r}	Cho (2009)
NU2	*h*$^-$ *leu1-32 ura4-d18 nup184-gfp::ura4*$^+$	
NU3	*h*$^-$ *leu1-32 ura4-d18 nup184-gfp::ura4*$^+$ /pREP41X-*pmt3*GG::LEU2	This study
NU4	*h*$^-$ *leu1-32 ura4-d18 nup211-gfp::ura4*$^+$	
NU5	*h*$^-$ *leu1-32 ura4-d18 nup211-gfp::ura4*$^+$ /pREP41X-*pmt3*GG::LEU2	This study
NU6	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZA69u-*nup184* full length	
NU7	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ2	This study
NU8	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ3	This study
NU9	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ4	This study
NU10	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ5	This study
NU11	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ6	This study

(continued)

NU12	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ7	This study
NU13	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ8	This study
NU14	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ9	This study
NU15	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ10	This study
NU16	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ11	This study
NU17	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ2-8	This study
NU18	*h*$^-$ *leu1-32 ura4-d18* Δ*nup184::kan*^{r} /pZU184Δ2-9	This study

Table 1. Strains used in this study (continued)

		Source
pREP41X	*nmt*-promoter medium strength, LEU2 marker pREP1 derivative, *bla* gene	Maundrell (1993)
pZA69u	*nmt*-promoter full strength, *ura4*+ marker, *bla* gene, pREP4 derivative	Yoon
pDW232	*ura4*$^+$ marker, Amp^r	Invitrogen
pDW234	pDW232 derivative, ARS deleted, *ura4*$^+$ marker, Amp^r	Yoon
pFA6a-kanMX4	pFA derivative, kanMX4 marker, Amp^r	
pFA6a-VC-kanMX6	pFA derivative, kanMX4 marker, Amp^r, VC tagging	Huh *et al.* (2007)

Ⅱ.4. Reagents

Products used for yeast culture are manufactured by BD (NJ, U.S.A.), Sigma (MO, U.S.A.), and MP (Eschwege, Germany). Agarose for DNA electrophoresis is produced by BioRad (CA, U.S.A.).

Isolation of chromosomal DNA, PCR purification, and gel extraction were done by using the kits which were manufactured by QIAGEN(Hilden, Germany). And DNA preparation was done by a kit from GeneAll (Seoul, Korea). The others are represented in each method of experiments.

Ⅱ.5. Primers and DNA sequence analysis

Primers contain 40-60% GC contents and avoid including repetitive AT sequences. We applied 1㎕ of 10μM primer to 50㎕ PCR reaction mixture for 0.2μM primer concentration. Table 3 represents primer sequences used in this study.

Ⅱ.6. Site-directed mutagenesis

QuikChangeⅡ XL Site-Directed Mutagenesis Kit was used to make site-specific mutations (Agilent Technologies, U.S.A.). First of all, the mutagenic oligonucleotide primers were designed individually according to the desired mutation. In this case, the target mutations were as follows; K729R, K1056R, K1132R, K1213R, and K1555R. The middle nucleotide A of codon usages of

lysine (AAG, AAA) in the SUMO consensus sequences of the *nup184* was substituted for nucleotide G by using mismatched sequences in the middle of each primer with 10~15 bases of correct sequence on both sides. Following thermal cycling step for mutant strand synthesis, the amplified products were treated with a restriction enzyme *Dpn*I in order to digest the parental pZU184Δ2 template and to select for mutation-containing synthesized pZU184Δ2. The *Dpn*I endonuclease (target sequence: 5'-G_m^6ATC-3') is specific for methylated and hemimethylated DNA. DNA isolated from the *E. coli* Top 10' is dam methylated and therefore susceptible to *Dpn*I digestion. The obtained vector DNA incorporating the desired mutations was then transformed into XL10-Gold ultracompetent cells. Among the isolated plamids, pZU184Δ2-3, Δ2-4, Δ2-5, Δ2-6, and Δ2-7 was sequenced to verify that selected clones contain correctly the designed mutations.

Ⅱ.7. Integration vector

After pD184-7th-pointed construct was prepared (based on a derivative of pDW232 lacking the ARS sequence, pDW234), the construct was digested with *Kpn*I for linearization and obtained DNA products were transformed into each target cell.

Table 3. Primers used in this study

Primer	Sequence (5'→3')	Restriction site contained
GFP-R5	TCCGTATGTTCCATCACC	
Primers used for DNA sequencing		
nmt-1	GGAATCCGATTGTCATTC	
NUq1	ACACATGGCATGGATGAAC	
NUq2	CTAGCTTCTGAAGGTCTTG	
NUq3	CGGCCATCGGTTTGCATC	
NUq4	GGCATACATGATTAACTC	
NUq5	CAGTTGCATATGTCCAAG	
NUq6	GTGGTTCAGGAGTTATCC	
NUq7	CATGGTCTCACTCATTG	
Primers used for serial deleted construction		
Nef40	ATCGTCGACGAGCTCTTGCACATGACG	*Sac*I, *Sal*I
Nef41	GTCCCAGGTACCAGTTTTTG	*Kpn*I
Nef42	AGGGATCCTCAGCAGCTTGTGCATCTAAC	*BamH*I
Nef43	ATCGGATCCTCAACCCTTCATTATTGA	*BamH*I
Nef44	ATCCTCGAGATGGGTGATTATTTA	*Xho*I
Nef45	ATCGGATCCTCATACAACAGGAGGATA	*BamH*I
Nef46	ATTGTCCCAGGTACCAGT	*Kpn*I
Nef47	GAGGATCCTCAAATAGTCGATGCCTC	*BamH*I
Nef48	GTGGATCCTCAAGCAAGTCTAATTTCTTG	*BamH*I

(continued)

Table 3. Primers used in this study (continued)

Sd4	GTGAATGGATCATATCTTAAGGCCGCAAT
Sd5	CCATACGTCTCAGGACTGATTTTGTTAAAAGGGCC
Sd6	GGCCCTTTTAACAAAATCAGTCCTGAGACGTATGG
Sd7	CCTTTAACGGTTAGGCTGGATGGGCTTAG
Sd8	CTAAGCCCATCCAGCCTAACCGTTAAAGG
Sd9	CACGTGCCTTTAGACATGAAAATGGAGAC
Sd10	GTCTCCATTTTCATGTCTAAAGGCACGTG
Sd11	GTTGAATTCGTCAGAGAAGACGCAACAC
Sd12	GTGTTGCGTCTTCTCTGACGAATTCAAC
Sd13	CTTGCTGAATTGAGGATTGAGATGCTG
Sd14	CAGCATCTCAATCCTCAATTCAGCAAG

Ⅱ.8. Transformations

Ⅱ.8.1. Transformation of *E. coli*

For the purpose of universal cloning, *E. coli* Top10' competent cells were used for transformation by *$CaCl_2$ method* (Sambrook & Russell, 2001). Following *E. coli* Top10' competent cells mixed with recombinant plasmid, the mixture was maintained under the 4℃ for 30 minutes. After that, 42℃ heat and ice-cold shock were conducted for 1 minute, respectively. And then regeneration was performed at 37℃ with 1㎖ of LB medium (20 volumes of the mixture) and shaking. Finally, cells were spread onto LA solid medium and incubated at 37℃ for about 16 hours.

Ⅱ.8.2. Transformation of *S. pombe*

The *S. pombe* transformation was routinely done using *classical lithium acetate (LiAc) method* according to the protocol (Sabatinos & Forsburg, 2010).

Yeast cells were grown in 50㎖ appropriate media to a density of 7-8x 10^6/㎖. After harvest the cells at 3000rpm for 2 minutes, cells were washed in 10ml TDW and were rotated down as before. Cells were resuspended at 1x 10^9/㎖ in 0.1M lithium acetate (yeast competent cells were made). 100㎕ competent cells were mixed with 3~5㎕ of recombinant plasmid and salmon sperm DNA (10㎎/㎖). Finally, the mixed sample was treated with 3 volumes of 50% PEG (50% polyethylene glycol4000 in 1X TE/LiAc) to 1 volume of

the mixture for 30 minutes. The blend was heatshocked at 42℃ for 10 minutes and the cell's pellets were obtained by centrifugation. The pellets were resuspended in TE and spread onto a selectable solid medium. They were at 29℃ until colonies were visible.

Ⅱ.9. Detection of protein

Ⅱ.9.1. Preparation of protein (*NP-40 based extraction method*)

Cells for Western blot analysis were grown in appropriated supplemented EMM medium to an exponential phase. Cells were harvested and washed in stop buffer and lysis buffer (Table 5). Acid-washed glass beads (0.5mm, Biospec) and 30$\mu\ell$ lysis buffer were added to the cell's pellet and mixed well (vortexing). Mini Beadbeater (Biospec product) was used to break the cell wall (4-8 repeats for each 30 seconds). Total cell extract was boiled in sample buffer (Bio-rad, U.S.A.) for 10 minutes and the supernatants were then obtained by centrifugation.

Ⅱ.9.2. Immunoprecipitation

Δ*nup184::kan*^{r} cells containing serially deleted pZU184 or pZU184Δ2-7 were grown in appropriated supplemented EMM medium to an exponential phase before being harvested. Following whole cell extraction, immunoprecipitation of protein A/G-tagged Nup184 was performed by using anti-GFP mouse antibody (abcam, England). The mixture was rotated for 2hrs at 4℃ with

washed protein A/G beads (Santacruze biotechnology, U.S.A.). The mixture was boiled in sample buffer and it was followed by centrifugation to get supernatant. And then SDS-PAGE was performed to separate denatured immunoprecipitates.

Ⅱ.9.3. Western blot analysis

For SDS-PAGE (SDS-Polyacrylamide Gel Electrophoresis), the denatured samples (including various soluble proteins) were subjected to SDS-PAGE at a constant voltage of 40 to run a 5% stacking gel and 120 to run a 7.5% resolving gel for 20 minutes and 1 hour 20 minutes, respectively. The proteins were transferred to a PVDF membrane (Amersham, GE healthcare, U.S.A.) where they were probed using antibodies specific to the target protein. For the membrane blocking, 5% skimmed milk (Scharlau Chemie, Barcelona, Spain) in TBS-T was used (Table 5). The blocked membrane was probed for the protein of interest with both a first antibody and a HRP-conjugated second antibody. The membrane containing antibodies modified protein was treated with an ECL detection solution (Amersham, GE healthcare, U.S.A.) and an emitted signal was detected under the Kodak image station 4000MM PRO (Kodak, U.S.A.).

Ⅱ.10. *S. pombe* genomic DNA isolation

Yeast cells were grown in appropriate EMM to a stationary phase. After harvesting the cells, 1ml of spheroplast buffer was added into the cell's pellet

for the cell wall disruption. Following steps were performed by using DNeasy Plant Mini Kit (Qiagen, Duesseldorf, Germany).

Ⅱ.11. Spot assay

Cells were grown in appropriate EMM at 29℃ before being diluted. The cells were resuspended in distilled water. 5㎕ of 10 fold serial dilutions of each yeast culture were spotted onto EMM and YES plates. Growth differences were recorded following incubation for 4~5 days at 29℃.

Ⅱ.12. *In situ* hybridization

Δ*nup184::kan*^{r} cells containing serially deleted pZU184 or pZU184Δ2-7 were grown in YES and EMM at 29℃ to an exponential phase before being harvested. For cell′s fixation, 6ml of 30% formaldehyde were added in 50ml cell culture and the mixture was incubated for 45 minutes at 29℃ with shaking. Following washing step with 0.3M PBS+Glycine, resuspended the cell′s pellet in spheroplast buffer to 1 x 10^{7}cell/ml. The culture was incubated at 37℃ for 1hr. Cells which were resuspended in SCE were applied onto poly-D-lysine coated slide. After incubation of the slides at 4℃ for 2hrs, the slides were submerged in -20℃ methanol for 2hrs. Following Drying methanol completely, the slides were washed with 2X SSC. And then the slides were applied with 10㎕ hybridization solution (Table 5) and incubated at 37℃ overnight. After washing step with 2X SSC, the slides was applied with 10㎕ rhodamine and incubated at 37℃ for 1hr. The incubated slides

were washed twice with 2X SSC at room temperature and treated with 12㎕ DAPI mounting medium and then sealed. Images were collected with an Olympus BX60 fluorescence microscope (Olympus, Japan).

Table 4. Composition of media for *S. pombe*

Yeast Extract (YE)	/ ℓ
0.5% Yeast extract	
3% Dextrose	
Yeast Extract + Supplements (YES)	/ ℓ
0.5% Yeast extract	
3% Dextrose	
Supplements	225㎍/㎖ adenine, leucine, ur
EMM (Edinburgh Minimal Medium)	/ ℓ
14.7mM Potassium hydrogen phthalate	
15.5mM Sodium phosphate dibasic	
93.5mM Ammonium chloride	
111mM Dextrose	2% (w/v)
50X Salts	2 ㎖
1000X Vitamins	1 ㎖
10000X Minerals	0.1 ㎖

(contin ed)

Table 4. Composition of media for *S. pombe* (continued)

	/ ℓ
	5
	0.7
670mM KCl	
14.1mM Na_2SO_4	2g
1000X Vitamin Stock	/ ℓ
4.2mM Pantothenic acid	
81.2mM Nicotinic acid	
55.5mM Inositol	
40.8μM Biotin	10㎎
10000X Mineral Stock	/ ℓ
80.9mM Boric acid	
23.7mM $MnSO_4$	
13.9mM $ZnSO_4 \cdot 7H_2O$	
7.40mM $FeCl_2 \cdot 6H_2O$	2g
2.47mM Molybdic acid	0.4g
6.02mM Kl	1g
1.60mM $CuSO_4 \cdot 5H_2O$	0.4g

47.6mM Citric acid	10g

Table 5. Buffers

S. pombe* transformation: *Lithium acetate (LiAc) method	
10X LiAc	1M Lithium Acetate pH7.5
10X TE	0.1M Tris-HCl pH7.0, 0.01M EDTA
50% PEG	50% Polyethylene glycol in 1X TE/LiAc
Chromosomal DNA isolation	
SCE (pH 5.6)	50mM Citrate acid, 50mM Na_2HPO_4, 1.2M Sorbitol, 40mM EDTA
Spheroplast buffer	Zymolase 20T (2.5㎎/㎖) in SCE buffer
Cell lysis buffer	
Composition	Protease and phosphatase inhibitor
6mM Na_2HPO_4	Protease inhibitor cocktail (incl. 2mM EDTA)
4mM NaH_2PO_4	4㎍/㎖ leupeptin
1% NP-40	0.1mM Na_3VO_4
150mM NaCl	1.3mM benzamidine
50mM NaF	1mM PMSF

(contin ed)

Table 5. Buffers (continued)

	% Triton X-100
	ilk in 1X TBS-T
Stop buffer	50mM NaF, 10mM NaN_3 in 1X PBS
Transfer buffer	25mM Tris base, 195mM Glycine, 20% Methanol, 0.2% SDS
Running buffer	25mM Tris base, 195mM Glycine, 0.1% SDS
***E. coli* Cracking**	
2X cracking buffer	0.2M NaOH, 0.5% SDS, 20% Sucrose
***In situ* hybridization**	
30% formaldehyde	30% Paraformaldehyde, 0.5mN NaOH in 1X PBS
Spheroplast buffer	Zymolase 100T in SCE
Hybridization solution	20X SSC, 50% Dextran sulfate, 2% BSA, Vanadyl complex, tRNA (1㎎/㎖), Oligo dT50
Fluorescin-Antidigoxigenin	0.1mM Tris pH7.5, 3mM NaCl, Antidioxigenin (200㎍/㎖), 2% BSA, 0.3% Triton X-100
DAPI mounting medium	DAPI (1㎎/㎖) in Glycerol

Ⅲ. Results

Ⅲ.1. Constructions of the strains for experiments

Ⅲ.1.1. Construction of pREP41X-*pmt3*GG plasmid

To test if SUMO is involved in mRNA export in fission yeast, we generated the haploid Δ*pmt3*::*kan*^{r} null strains (*pmt3*$^{+}$; the fission yeast gene encoding SUMO). These strains, however, grew very poorly and showed severe morphological defects. For detection of SUMO, these strains should be transformed with plasmid expressing Myc-tagged *pmt3*$^{+}$ under the control of *nmt*-promoter. We wanted to make the strain harboring both Δ*pmt3*::*kan*^{r} and *nup184-gfp*::*ura4*$^{+}$ alleles and plasmid expressing Myc-tagged *pmt3*$^{+}$, to figure out whether the Nup184 is sumoylated or not. The plasmid expressing *pmt3*$^{+}$ and *nup184-gfp* allele should have different marker to put together into one cell. However, because *nup184-gfp* strain was already constructed using *ura4*$^{+}$ and pREP42X-*pmt3*GG were marked by using *ura4*$^{+}$, the selectable marker gene of the plasmid expressing Myc-tagged *pmt3*$^{+}$ had to be replaced by another marker gene.

So, we planned to construct pREP41X-*pmt3*GG which have LEU2 marker gene. pREP41X-*pmt3*GG was constructed as follows. *Pst*I site upstream of P*nmt* and *Sac*I site downstream of T*nmt* was used to generate P*nmt-pmt3*GG-

T*nmt* DNA fragment from pREP42X-*pmt3*GG. And this DNA fragment was inserted into pREP41X digested with *Pst*I and *Sac*I. Finally, we could get pREP41X-*pmt3*GG plasmid that has LEU2 marker and expresses *pmt3*GG under the control of the mid-strength thiamine-repressible *nmt*-promoter on pREP41X.

Ⅲ.1.2. Construction of PUX2 strain by Random Spore Analysis (Δ*pmt3*::*kan*^{r} *nup184-gfp*::*ura4*$^+$ /41X-*pmt3*GG)

To investigate whether the Nup184 is sumoylated or not, Δ*pmt3*::*kan*^{r} *nup184-gfp*::*ura4*$^+$ /pREP41X-*pmt3*GG strain was necessary. This strain was made by using Random Spore Analysis (RSA). RSA allows much more spores to be examined than tetrad analysis and thus facilitates rapid recombination mapping and strain constructions.

First, pREP41X-*pmt3*GG was transformed into Δ*pmt3*::*kan*^{r} heterozygous diploid. The selected Leu$^+$ transformants of h^+/h^{90} heterozygous diploids were sporulated on ME medium and screened for growth both on E-L+B_1 and YE_{G418} plates. These cells contain both the plasmid and Δ*pmt3*::*kan*^{r} null allele (kanamycine resistance). Selected cells were crossed with h$^+$ strain to separate h^- cells.

Second, isolated cells (h^+ Δ*pmt3*::*kan*^{r} /pREP41X-*pmt3*GG) were crossed with h^- *nup184-gfp*::*ura4*$^+$ cells on ME medium and the spores germinated on

YES plate. And we screened out the cells that could grow on all of E-L, U+B_1 and YE_{G418} plates. The selected cells were mated with wild type AY217 (h^-) and 216 (h^+) strains to separate h^+ and h^- cells, respectively. Finally, we could obtained the strain, PUX2, harboring the specific genotype: Δ*pmt3*::*kan*^{r} *nup184-gfp*::*ura4*$^+$ /pREP41X-*pmt3*GG.

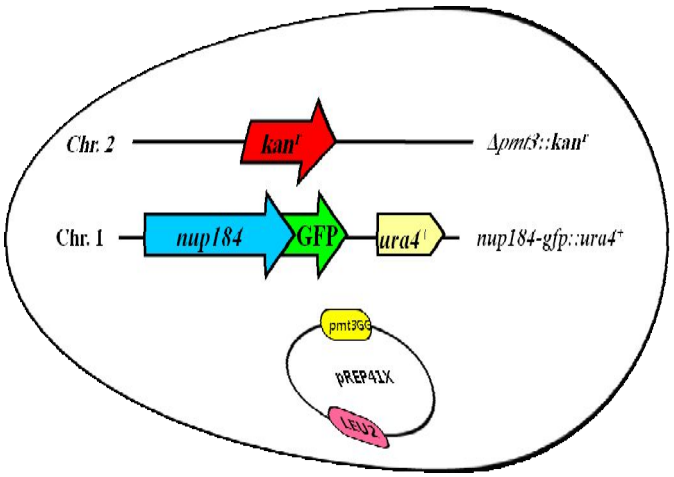


Figure 1. Schematic diagram of the PUX2 strain

Ⅲ.2. Analysis of *nup184* serial truncated mutants

Ⅲ.2.1. Phenotype of *nup184* serial truncated mutants

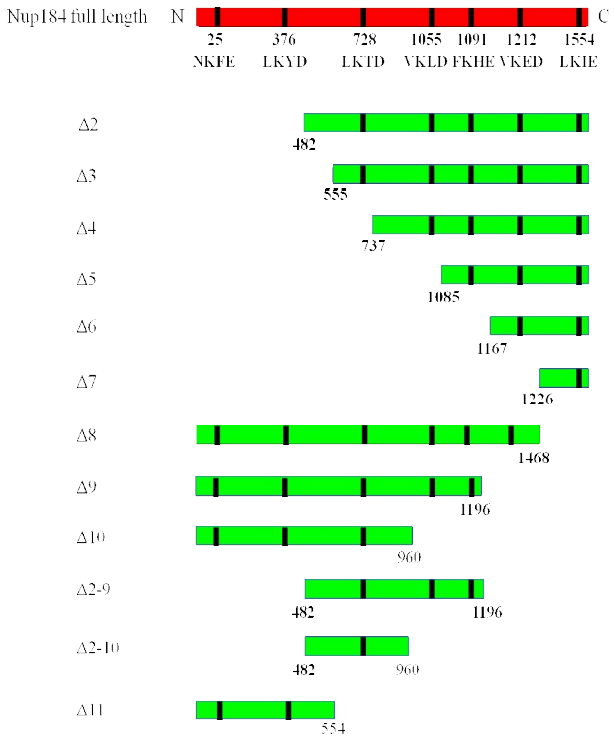
nup184 serial deleted constructs (pZU184, pZU184Δ2~10) were made

previously using PCR-based method (Moon, 2010). Positions for serial truncations were based on seven SUMO consensus sequences (ψKXE/D, where ψ represents large hydrophobic residue) known as the only spots where SUMO can conjugate. These constructs have truncated Nup184 expressed from the full-strength thiamine-repressible *nmt*-promoter on pZA69u. These plasmids were transformed to *nup184* null mutant, where the entire *nup184* ORF is replaced with a kanamycine resistant marker (Jo, 2009). Schematic diagram of Figure 2 represents the sequential truncated constructs of Nup184.

Although Nup184 is not essential, *nup184* null mutant grows feebly on the nutrient-rich medium (YES) in contradistinction to PMG or EMM minimal media (Jo, 2009). To know whether our constructs of serial truncated mutant complement the growth retardation on YES medium, growth test was performed by using spot assay (Figure 4). These mutants were spotted in 10-fold serial dilutions onto YES, PMG, and EMM plates, and incubated 4~5 days at 29℃ before being photographed. pZU184 full length and pZU184Δ2 complemented growth defect on YES as compared with Δ*nup184*::*kan*^{r} strain that transformed with other pZU184 series vector (Figure 4). pZU184Δ4, Δ5, Δ6, Δ7, and Δ10 in common with Δ3 have growth retardation on YES medium by comparison with PMG and EMM minimal media (data not shown).

Actually, *nup184* sequence listed in *Schizosaccharomyces pombe* GeneDB (hosted by the Sanger Institute) was different from our sequenced data obtained from wild type 972: ORF of Nup184 registered in *S. pombe* GeneDB was shorter than that predicted by our sequence data. Our Nup184 was longer

about 66 amino acids than that of *S. pombe* GeneDB. Thus we refer our full length and that of *S. pombe* GeneDB to tailed Nup184 and non-tailed Nup184, respectively. Non-tailed Nup184 did not complement the growth retardation on YES, while tailed Nup184 (pZU184 full length) did. Therefore, we thought that the tailed portion of Nup184 might be important to the function of



Nup184.

Figure 2. Schematic diagram showing the *nup184* serial truncated constructs

NKFE (25), LKYD (376), LKTD (728), VKLD (1055), FKHE (1091), VKED (1212), LKIE (1554) stand for SUMO consensus sequences

(ψKXE/D, where ψ represents large hydrophobic residue).

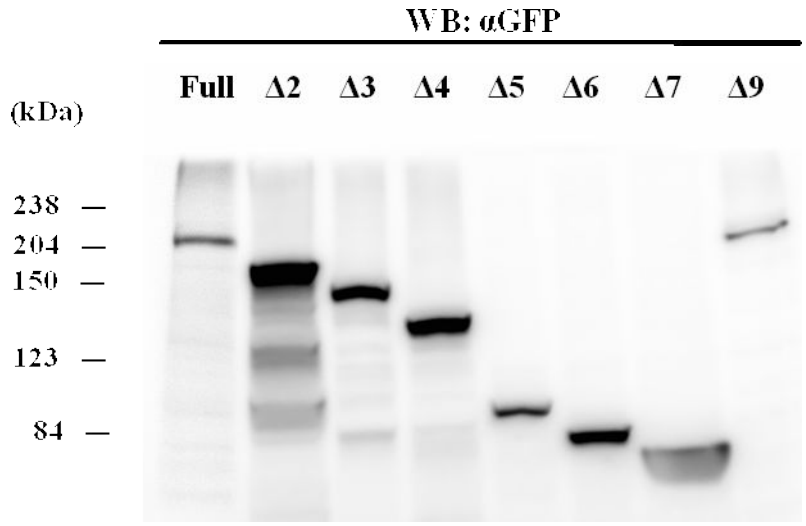


Figure 3. Expression of serial deleted Nup184p::GFP

Whole cell extracts which was prepared from the *nup184-gfp::ura4*$^{+}$ cells transformed with plasmids overexpressing serial truncated Nup184 were subject to Western blot analysis with Anti-GFP antibody.

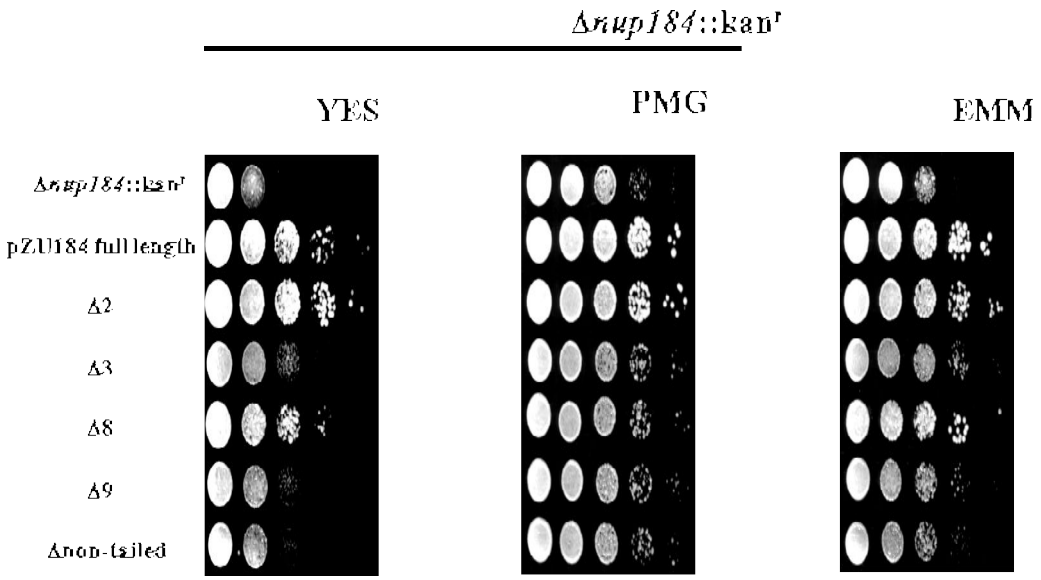


Figure 4. Growth test for *nup184* serial truncated constructs by spot assay

Mutants of serial truncated *nup184* show growth retardation in nutrient rich medium (YES). Yeast Δ*nup184*::*kan*^{r} cells were transformed with a vector expressing serial deleted Nup184. 5$\mu\ell$ of 10 fold serially diluted cultures were spotted on complete medium (YES) and minimal media (PMG and EMM) and incubated at 29℃ for 4-5 days before being photographed.

Ⅲ.2.2. SUMO modification of *nup184* serial truncated mutants

To know whether Nup184 is SUMOylation or not, we performed immunoprecipitation-Western blot analysis (IP-WB). Because the constructed strains are *nup184-gfp::ura4*$^{+}$/pZU184 series /pREP41X-Myc-*pmt3*GG (mature form of *Pmt3*), GFP-tagged Nup184 and Myc-tagged Pmt3 (SUMO) was over-expressed, respectively. Immunoprecipitation was done with an anti-GFP mouse antibody for the Nup184 precipitation. The immunoprecipitated fractions were analyzed by Western blotting with an anti-GFP goat antibody or an anti-Myc mouse antibody. If Nup184 interacts with Pmt3 *in vivo*, membrane which is blotted with immunoprecipitates would show higher molecular weight bands than correct sized bands of Nup184 alone in Western blot analysis with an anti-GFP. At the same time, membrane which is applied with the same samples mentioned above (immunoprecipitates) might represent a patterns in Western blot analysis with an anti-Myc, matching the result of Western blotting with an anti-GFP. Moreover, we wanted to know the spot where conjugation of SUMOs mainly occurs among the five putative SUMO consensus sequences in Nup184 by this experiments.

For discovering that the Nup184 protein is actually modified by SUMO, we primarily wished to test the levels of SUMO-conjugates in the Nup184 full length, Δ2, and Nup211 (negative control) strains. Anti-Nup184 samples were prepared as described in the Materials and Methods. Figure 5 gives that the Nup184 full length and Δ2 interact with Pmt3 as observed by the upper bands

present in lane2 and 3 of right pannel, but which is not presents in the negative control (lane1, right panel).

After that, we prepared the immunoprecipitates of more serial deleted mutants of Nup184 (Δ3, Δ4, Δ5, Δ6, Δ9, and Δ10). Analysis of serial truncated mutant cells shows higher molecular bands than original mutant Nup184 bands when Western blotting was done with both anti-GFP and anti-Myc, indicating that the up smeared Nup184 bands is due to Pmt3 modification (Figure 6). Specifically, Δ9 and Δ10 was severely SUMOylated as compared with the others (lane8 and 9). This result proposed that rear end of the Nup184 might play a role in controlling SUMO modification negatively.

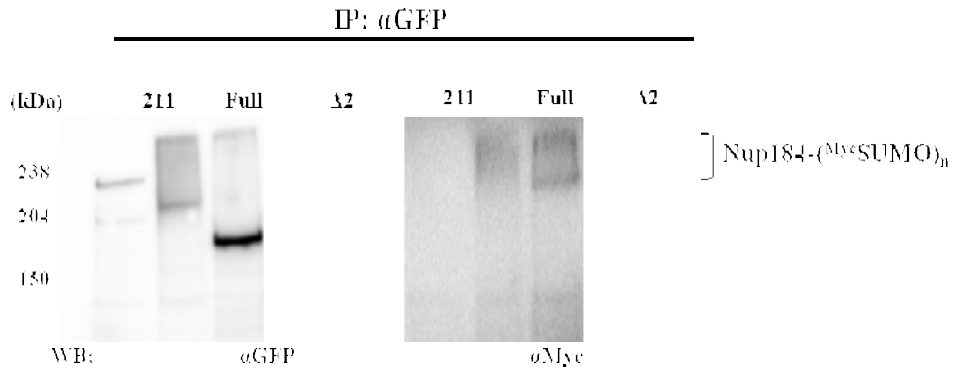


Figure 5. Interaction of Nup184 with Pmt3

Whole cell extracts prepared from cells overexpressing both GFP-Nup184 and Myc-Pmt3. The immunoblotted membranes were incubated in TN_TX buffer for 2hrs with appropriate concentration of each antibody. Immunoprecipitation (IP) and Western blot (WB) analyses with anti-GFP antibody recognize the slow-migrating bands as SUMO conjugated forms of Nup184 (left panel). SUMOylation of Nup184 was shown by Western blotting with anti-Myc antibody (right panel).

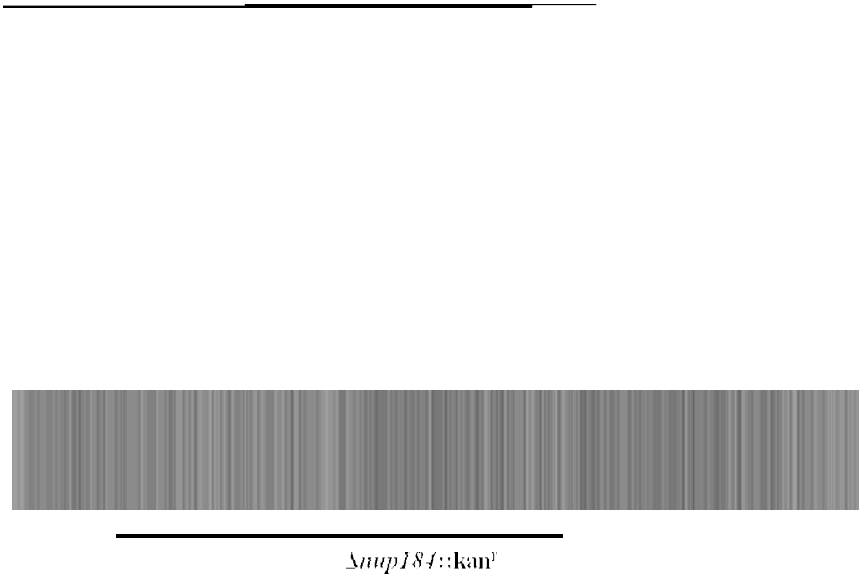


Figure 6. Interaction of sequencial truncated Nup184 with Pmt3

Immunoprecipitation of whole cell extracts which was prepared from cells either overexpressing GFP-Nup184 and Myc-Pmt3 was done with anti-GFP antibody. The immunoprecipitates were anlyzed by Western blotting with both an anti-GFP (top) and an anti-Myc antinody (bottom). Nup184 might interact with SUMO *in vivo*.

Ⅲ.3. *nup184* point mutants

Ⅲ.3.1. Construction of *nup184* point mutants

Nup184 protein has seven putative SUMO consensus sequences as shown in the figure 8 (black vertical bars). We constructed five site-directed mutants to further check which could complement the growth retardation and defects of mRNA export in complete medium (YES), and which region is mainly conjugated for SUMO. Figure 7 represents schematic diagram of five point mutated constructs.

Because the size of pZU184Δ2 plmasmid was large (12.5kb), which was template of site-directed mutagenesis, QuikChangeⅡ XL Site-Directed Mutagenesis Kit was used to make site-specific mutation (Agilent Technologies, U.S.A.). First of all, the mutagenic oligonucleotide primers were designed individually according to the desired mutation. In this case, the target mutation were as follows; K729R, K1056R, K1132R, K1213R, and K1555R. The middle nucleotide A in lysine (K) of SUMO consensus sequences (AAG or AAA) was substituted for nucleotide G, resulting in arginine (R), by using mismatched sequences in the middle of each primer with 10~15 bases of correct sequence on both sides. Following thermal cycling step for mutant strand synthesis, the amplified products were treated with restriction enzyme *Dpn*I in order to digest the parental pZU184Δ2 template and to select for mutation-containing synthesized pZU184Δ2. The

*Dpn*I endonuclease (target sequence: 5'-G_m^6ATC-3') is specific for methylated and hemimethylated DNA. DNA isolated from the *E. coli* Top 10' is dam methylated and therefore susceptible to *Dpn*I digestion. The obtained vector DNA incorporating the desired mutations is then transformed into XL10-Gold ultracompetent cells. The number of colony was about 500. Among the isolated plamids, pZU184Δ2-3, Δ2-4, Δ2-5, Δ2-6, and Δ2-7 was sequenced to verify that the selected clones contain correctly the designed mutations.

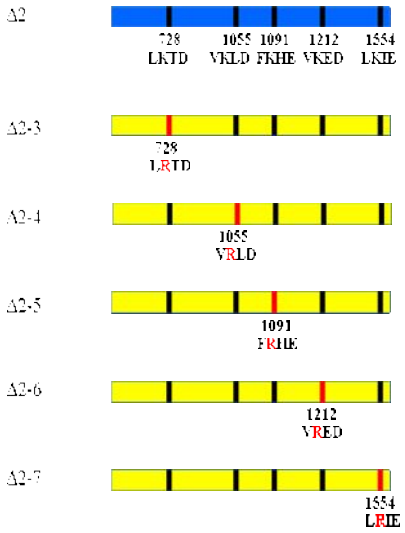


Figure 7. Schematic diagram showing the *nup184* point mutated constructs

Nup184 point mutants prepared from Nup184Δ2 truncated construct. Capital letters stand for SUMO consensus sequences (ψKXE/D, where ψ represents large hydrophobic residue). Alphabets colored red indicate that amino acids substitute for lysine (K) to arginine (R).

Ⅲ.3.2. Analysis of phenotype of *nup184* point mutants

To examine the complementation of these constructs for growth defect of *nup184* null cells, spot assay was performed on the media of YES, PMG, and EMM. Figure 8 gives the results of these. Among the five constructs, pZU184Δ2-3, Δ2-4, Δ2-5, and Δ2-6 complemented the growth defects on complete media. However, in the case of pZU184Δ2-7, growth retardation was severe compared with the others, suggesting that the last SUMO consensus sequence of Nup184 might have a role in competence of complementation of this gene. The reason why growth pattern of EMM looks similar to that of YES is due to difference of growth velocity between complete and minimal medium. It is clearly seen on size of colonies in lowest dilution spot.

Morphology of the pZU184Δ2-3, Δ2-4, Δ2-5, and Δ2-6 cells were relatively regular than Δ2-7 when they were observed under the light microscope. Shape of Δ2-7 cells were much longer than the other, corresponding with growth retardation. In addition, irregularly-branched cells were shown in some of the mutant cells. Furthermore, pZU184Δ2-7 strain has a few erratic vesicles inside the cell. The vesicles seem to be colored black under the microscope.

The Δ2-7 mutant cells, which were thought to be defective in mRNA export, were subject to one of samples used for *in situ* hybridization. Image of fluorescent mRNA merged with images for DAPI for comparing localization

of nucleus. mRNA of the Δ2-7 was severely accumulated in the cell's nucleus (figure10, right panel, the bottom).

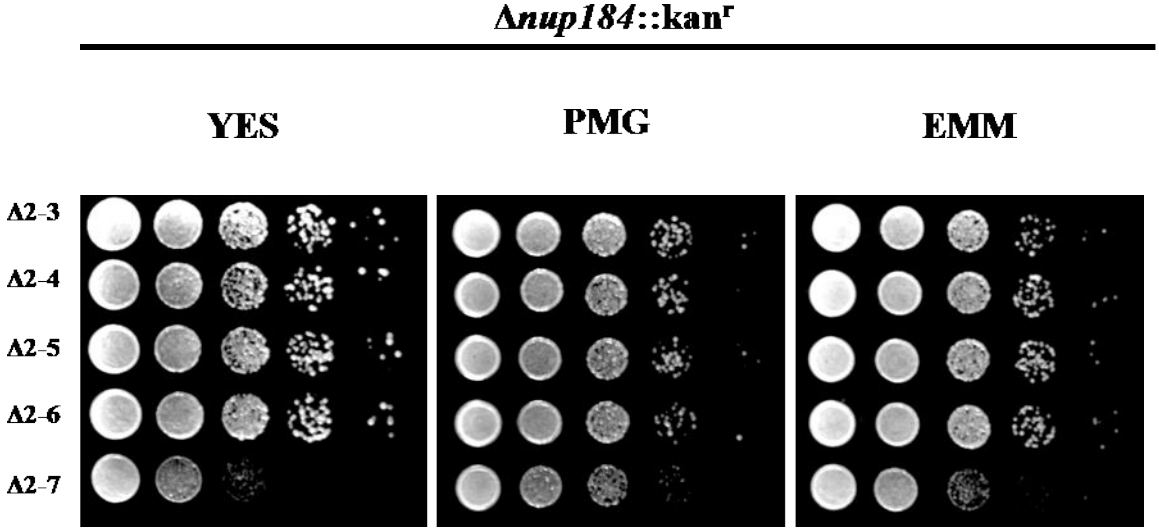


Figure 8. Growth test for *nup184* point mutants

Point mutants of *nup184* show growth defects in complete medium (YES). Yeast Δ*nup184*::*kan*^{r} cells were transformed with pZU184Δ2-3, Δ2-4, Δ2-5, Δ2-6, and Δ2-7. 5$\mu\ell$ of 10 fold serial dilutions were spotted on complete medium (YES) and minimal media (PMG and EMM) and grown at 29℃ for 4-5 days before being photographed.

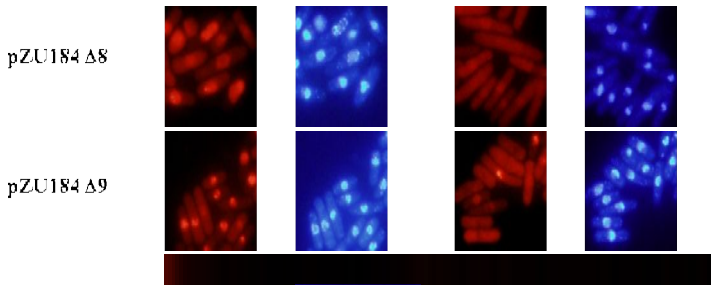


Figure 9. Poly(A)$^+$ localization in *nup184* sequential truncated and point mutant cells

The cells were grown at 29℃ to exponential phase and stained for poly(A)$^+$ RNA. Coincident DAPI staining is shown in each on the right. The growth retardation of the *nup184* sequential truncated and point mutants were correlated with mRNA export defects.

Ⅳ. Discussion

In eukaryotes, mRNA export from the nucleus is occurred through a specialized pathway by comparison with export of other RNAs. It is considerably important to understand mechanisms of mRNA export as mRNA is like a blueprint for genes of living things.

The *nup184* gene in fission yeast *S. pombe* is located in chromosome 1 and encodes a nucleoporin, which is a component of nuclear pore complex (NPC) that is massive protein assemblies, perforating the nuclear envelope (NE). Even though it is not essential for growth, Δ*nup184* null mutant shows retardations of both growth and mRNA export in complete medium (YES). Whalen *et al.* (1999) discussed that the *nup184* null allele is synthetically lethal with *rae1-167,* important gene related to mRNA export. It is thought to be modified by SUMO, which is acronym for a small ubiquitin-like modifier, owing to high molecular weight patterns of Nup184. Sure enough, Nup184 has seven supposed SUMO consensus sequences as follows; 728LKTD, 1055VKLD, 1091FKHE, 1212VKED, and 1554LKIE.

We wanted to know whether Nup184 actually SUMOylated or not and if so, what the function of SUMO modification of Nup184p is and which region is required for SUMOylation and growth complementation. To solve these questions, we constructed two strains and 15 vectors as follows: 1. Δ*nup184*::*kan*^{r} null mutant and *h*$^{-}$ Δ*pmt3*::*kan*^{r} *nup184-gfp*::*ura4*$^{+}$ /pREP41X-

*pmt3*GG cells; 2. pZU184 full length, pZU184Δ2~10 (serial truncated constructs), and pZU184Δ2-3~7 (point mutated constructs). The schematic diagrams of sequential truncated and point mutated constructs are shown in Figure 2 and 7.

First of all, to test which construct among the serial truncated mutants could complement the growth defect, the cells were spotted onto YES, PMG, and EMM plates. As you can see in Figure 4, the constructs of Δ3, Δ2-8, Δ9, and Δ10 did not complement growth defects. This result correspond with observable defects in mRNA export, seeing that poly(A)$^+$ RNA is accumulated in the nucleus (Figure 9). In summary, these results suggest that growth retardation of *nup184* mutants is accompanied by their mRNA export.

The five point mutants (pZU184Δ2-3, Δ2-4, Δ2-5, Δ2-6, and Δ2-7) were generated to further check which region is important for complementation of the growth retardation and is mainly conjugated with SUMO. Figure 8 gives the results of growth complementation. The first four constructs among the five complemented the growth defect on nutrient rich medium (YES). However, growth retardation of Δ2-7 was relatively severe to others (the first four constructs), suggesting that the last portion of *nup184* might have an important role function of this gene. Moreover, in Δ2-7 cell, there was detectable nucleic accumulation of poly(A)$^+$ RNA (Figure 9, the bottom). The growth pattern of EMM looks similar to that of YES is due to difference of growth rate between complete and minimal medium. We can assume this by observing the size of colonies in the lowest dilution spot. Collectively, these

results presented above suggested that the rear region of the Nup184 might have important functions for the growth and mRNA export.

As we mentioned in introduction, *nup184* sequence listed in *S. pombe* GeneDB (hosted by the Sanger Institute) was different from our sequenced data obtained from wild type 972: the predicted ORF registered in *S. pombe* GeneDB was shorter than our ORF. Thus we named our full length of Nup184 and that of *S. pombe* GeneDB as tailed Nup184 and non-tailed Nup184, respectively. Non-tailed nup184 strain, which was constructed by using PCR method, did not complement the growth on YES by comparing with tailed Nup184 (pZU184 full length). Therefore, we also thought that the tailed part of Nup184 might be significant to the gene's complementation (Figure 4, right panel, the bottom).

The serial deleted Nup184s and high molecular weight forms of these mutants were observed in Figure 3. Whole cell extracts, which was prepared from the Δ*nup184*::*kan*^{r} cells transformed with a plasmids overexpressing serial deleted Nup184, were subject to Western blot analysis with anti-GFP goat antibody.

To confirm that smeared bands are caused by SUMOylation of Nup184, we carried out immunoprecipitation-Western blot analysis (IP-WB). Subjects are the serial truncated mutants as referred to earlier. Immunoprecipitation was conducted with an anti-GFP mouse antibody for the Nup184 precipitation. The immunoprecipitated fractions were analyzed by Western blotting with

both an anti-GFP goat antibody and an anti-Myc mouse antibody. Figure 5 and 6 show that the Nup184 interact with SUMO as observed by the high molecular weight bands. Especially, Δ9 and Δ10 were severely SUMOylated compared to others, suggesting that the rear end of the Nup184 might play a role in controlling SUMO modification negatively (Figure 6, lane8 and 9).

In this study, we have shown that serially deleted or point mutated *nup184* (Δ3, Δ2-8, Δ9, Δ10, and Δ2-7) represented growth retardation in nutrient-rich medium (YES) and was unsuccessful in exporting mRNA to cytoplasm. Specifically, C-terminal deleted mutants and Δ2-7 which has point mutated last SUMO consensus sequence were severely SUMOylated, moreover, localizaton of poly(A)$^+$ RNA was accumulated in their nucleus. These results proposed that C-terminal end of the Nup184 might play a important role in controlling SUMO modification negatively, gene complementation, and poly(A)$^+$ RNA exporting.

References

Alfred C.O. Vertegaal. (2010) SUMO chains: polymeric signals. *Biochem. Soc. Trans.* 38, 46-49

Alwin Kohler and Ed Hurt. (2007) Exporting RNA from the nucleus to the cytoplasm. *NATURE* 8, 761-773

Andrew Skilton, Jenny C. Y. Ho, Brenda Mercer, Emily Outwin, and Felicity Z. Watts. (2009) SUMO chain formation is required for response to replication arrest in *S. pombe*. *PLos ONE* 4, e6750

Charles N Cole and John J Scarcelli. (2006) Transport of messenger RNA from the nucleus to the cytoplasm. *Curr Opin Cell Biol*. 18, 299-306

Gandhi Theerthagiri, Nathalie Eisenhardt, Heinz Schwarz, and Wolfram Antonin. (2010) The nucleoporin Nup188 controls passage of membrane proteins across the nuclear pore complex. *J. Cell Biol.* 189, 1129-1142

Jae-Hyuk Yu, Zsuzsanna Hamari, Kap-Hoon Han, Jeong-Ah Seo, YazmidReyes-Dominguez, and Claudio Scazzocchio. (2004) Double-joint PCR: a PCR-based molecular tool for gene manipulations in filamentous fungi. *Fungal Genetics and Biol.* 41, 973-981

Jenny C. Y. Ho, Nicholas J. Warr, Harumi Shimizu, and Felicity Z. Watts. (2001) SUMO modification of Rad22, the Schizosaccharomyces pombe homologue of the recombination protein Rad52. *Nucleic Acids*

Res. 29, 41179-4186

Jin A Shin, Eun Shik Cho, Hyun Soo Kim, Jenny C.Y. Ho, Felicity Z. Watts, Sang Dai Park, and Yeun Kyu Jang. (2005) SUMO modification is involved in the maintenance of heterochromatin stability in fission yeast. *Mol. Cell*. 19, 817-828

Jin Ho Yoon, William A. Whalen, Anekella Bharathi, Rulong Shen, and Ravi Dhar. (1997) Npp106p, a *Schizosaccharomyces pombe* nuclear similar to *Saccharomyces cerevisiae* Nic96p, functionally interacts with Rae1p in mRNA export. *Mol. Cell. Biol.* 17, 7047-7060

Kevin A. WILKINSON and Jeremy M. HENLEY. (2010) Mechanisms, regulation and consequences of protein SUMOylation. *Biochem. J.* 428, 133-145

Liya Ge, Xiao-Tao Wang, Swee Ngin Tan, Heng Hang Tsai, Jean W.H. Yong, and Lin Hua. (2010) A novel method of protein extraction from yeast using ionic liquid solution. *Talanta* 81, 1861-1864

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

Min-Kyung Sung and Won-Ki Huh. (2007) Bimolecular fluorescence complementation analysis system for *in vivo* detection of protein-protein interaction in *Saccharomyces cerevisiae*. *Yeast* 24, 767-775

Murry Stewart. (2007) Molecular mechanism of the nuclear protein import cycle. *NATURE* 8, 195-208

Patrizia Vinciguerra and Francoise Stutz. (2004) mRNA export: an assembly line from genes to nuclear pores. *Curr Opin Cell Biol*. 16, 285-292

Roderick Y. H. Lim, Ueli Aebi, and Birthe Fahrenkrog. (2008) Towards reconciling structure and function in the nuclear pore complex. *Histochem Cell Biol*. 129, 105-116

Thomas U Schwartz. (2005) Modularity within the architecture of the nuclear pore complex. *Curr Opin Struct Biol*. 15, 221-226

국문초록

분열 효모인 *Schizosaccharomyces pombe*의 *nup184* 유전자는 핵공 복합체의 구성요소인 핵공 단백질이다. 핵공 복합체는 단백질로 이루어진 거대 조립체로써 핵막을 통과하고 있어 핵과 세포질 사이의 물질 이동에 관여한다. *nup184* 유전자는 균주의 생장에 필수적이지는 않지만 이 유전자의 결실 돌연변이는 완전배지에서 낮은 생장률을 보이고 mRNA의 세포질 수송에 결함을 보인다. *nup184*의 서열에는 총 7개의 SUMO consensus 서열이 존재하는데 이 서열은 SUMO 단백질이 결합할 수 있다. *nup184* 내에 존재하는 SUMO consensus sequences는 다음과 같다; 728LKTD, 1055VKLD, 1091FKHE, 1212VKED, 1554LKIE. SUMO는 97개의 아미노산으로 이루어진 작은 단백질이고 유비퀴틴과 유사하다. SUMO는 *S. pombe*와 *S. cerevisiae*에서 각각 *pmt3*와 *smt3*에 의해 암호화 되어있다. 이 단백질은 번역 후 변형과정에 관여하는 단백질로써 목표 단백질의 라이신 잔기에 결합하여 그 기능을 조절한다. SUMO 변형은 (SUMOylation) 세포 내 기능을 조절하는데 이에는 전사, DNA 손상 반응, 세포 주기, 핵 수송 등이 포함된다. 이 연구에서 우리는 Nup184 단백질이 SUMOylation 되는지에 관한 연구, 만약 그렇다면, SUMOylation이 Nup184의 기능에 미치는 영향에 관한 연구 등을 진행할 것이다.