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A Conditional Lethal Mutation of a Nucleoporin Gene, *NUP49* in *Saccharomyces cerevisiae*

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Conditional lethal mutation *nup49-1* of a nuclear pore complex component gene was constructed in *Saccharomyces cerevisiae*. This mutation deleted one third of the essential *NUP49* gene at the carboxy-terminal, but retained 13 repeats of the highly conserved GLFG domain. The *nup49-1* mutant strain was viable with a slow-growth phenotype, indicating that the C-terminal is dispensable at normal growth temperature. This strain exhibited both temperature-sensitivity at 37°C and cold-sensitivity at 16°C. Temperature shift experiments revealed that the arrest phenotype at 37°C was random in the cell division cycle. The *nup49-1* mutation was tested to be recessive and is expected to be useful for the functional analysis of nuclear pore complex proteins as well as for studies of nuclear transport systems.

Key words: Nucleoporin gene, yeast, temperature-sensitivity, nuclear pore

In eukaryotic cells, the nucleus is separated from the cytoplasm by the nuclear envelope. The import of nuclear proteins synthesized in the cytoplasm and the export of mRNA, tRNA, and preribosomal subunits out of the nucleus are essential processes (6). These nucleocytoplasmic transports occur through the nuclear pore complexes (NPC) embedded in the nuclear envelope. Movement of materials across the nuclear envelope has been reported to show both specificity and directionality (11, 12). As revealed by electron microscopic analysis, the NPC is 120 nm in diameter and is composed of a cytoplasmic and a nuclear ring with octagonal symmetry, eight spokes, and a central particle referred as the plug or transporter (6).

The protein components of NPC (nucleoporin) are estimated to be more than 100 different polypeptides, but only a limited number of neucleoporins have been identified in several organisms (12). Mammalian p62, its yeast homolog Nup 1, and yeast Nsp1 comprise a group of nucleoporins which share multiple 'FSFG' peptides (2, 18). The second class of nucleoporins are characterized by the repeat motif 'GLFG'. Nup49p, Nup100, Nup 116p, and Nup145p identified in *Saccharomyces cerevisiae* are included in this class (10, 16). Although these repetitive domains are highly conserved among known nucleoporins, recently identified nucleoporins Nup133 and Nic96 lack any obvious repeats (4, 7). Since the identified neucleoporin genes account for only a small percentage of the total members, more genes with distinct features could be isolated through genetic screens or biochemical purifications.

Most known nucleoporins show nuclear pore localization by immunofluorescence experiments or have genetic or biochemical interactions with previously identified nucleoporins. However, the precise functions of each protein in nucleocytoplasmic transport remain unknown. The conditional lethal mutation of *NSP1*, which was mapped at the C-terminal domain, caused an accumulation of nucleolar proteins in cytoplasm (10). The removal of the repetitive FSFG domains residing in the middle of the Nsp1 protein did not affect its function, implying that the presence of conserved 'FSFG' or 'GLFG' repeats may not confer any discrete functions to these proteins. Disruptants of the nonessential nucleoporin genes *NUP116* or *NUP145* have been shown to be defective in poly(A)$^+$ RNA export (5, 17).

In this paper, we describe the construction of a conditional mutation of an essential nucleoporin gene, *NUP49*. This mutation deleted one third of the Nup 49 C-terminal and caused both temperature-sensitivity and cold-sensitivity. We expect that this mutation will be useful for studies of nucleocytoplasmic transport mechanisms and for genetic approaches to

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identifying new neucleoporin genes.

Materials and Methods

Strains, media, and genetic analysis

S. cerevisiae strain used for gene replacement is a diploid strain F762/F763 constructed by crossing strain F762 (*MATa ura3-52 leu2-3,112 his4-34*) and strain F763 (*MATα ura3-52 leu2-3, 112 his4-34*). Diploids were isolated by manipulating zygotes under the microscope and were confirmed by testing sporulation. Haploid yeast strains F760 (*MATa ura 3-52 lys2-208 trp1-1*) and 6947-1D (*MATa leu2-3 lys1-1 met3*) were used for dominance test. Yeast media were prepared as described by Sherman *et al.* (14). SC-Leu plates consisted of YNB with all amino acids except for leucine.

Escherichia coli HB101 (1) was used for bacterial transformation and plasmid propagation. Bacterial media were made as described by Sambrook *et al.* (13).

Transformation and DNA manipulation techniques

Yeast transformation was carried out by the lithium acetate method developed by Ito *et al.* (8) using 50 μg of sonicated calf thymus DNA (Sigma Inc.) per transformation as carrier. *E. coli* transformation was performed by the calcium chloride procedure of Mandel and Higa (9). Rapid isolation of plasmid DNA was done by the modified alkaline lysis method (13). Restriction endonuclease analysis and agarose gel electrophoresis were carried out as described by Sambrook *et al.* (13).

Mutant plasmid construction and gene replacement

Plasmid pJI203 carries the 9 kb *Pvu*II-*Sal*I DNA fragment, including the wild type *NUP49* gene, inserted at *Sma*I and *Sal*I sites of plasmid YEp24. To construct a C-terminal detetion mutation of *NUP49*, the *Sal*I-*Xho*I fragment haboring *LEU2* was isolated from plasmid YEp13 and ligated into the *Bgl*II digested pJI203. Cohesive ends were filled with Klenow enzyme and blunt ends were ligated.

Gene replacement was carried out by tranforming a diploid yeast strain F762/F763 with a 5.7 kb *Pvu*II fragment of the mutant plasmid. This DNA fragment contains *nup49::LEU2* mutation.

Results and Discussion

Construction of the *nup49-1* mutation

The yeast Nup49 protein exhibits 13 repeats of the conserved GLFG domain in the amino-terminal half (14-240 residues) of its 473 amino acids. Its carboxy-terminal half is composed of helix-forming residues. The second half of this domain shows a heptad repeat pattern with hydrophobic amino acids at positions 1 and 4 of a 7-residue long repeating sequence, which is similar to the heptad repeats within the Nsp1 carboxy-terminal domain (6). This part of the protein could be involved in coiled coil protein interaction. Since evidence has been presented that the Nup49 protein interacts with the Nsp1 protein and Nsp1 has been already reported to exert its essential function by its carboxy-terminal domain (7, 10, 18), we were interested in constructing a Nup49 truncation mutation by deleting the carboxy-terminal domain.

The *NUP49* gene was first identified by its protein cross-reactivity with monoclonal antibodies against a rat liver NPC fraction (16). The gene was known to be located between *KEM1* and *ROK1* genes on the left arm of chromosome VII (Fig. 1) (15). Plasmids pJI203 and pKL6 carrying *NUP49* were isolated in our laboratory in the process of cloning *ROK1* (15). We used plasmid pJI203 for the construction of a *nup49* deletion mutation. The internal *Bgl*II fragment in NUP49 as replaced with *LEU2* in such a way that the *NUP49* open reading frame ends at the stop codon present in the beginning of the *LEU2* gene (Fig. 1). The Nup49 protein is truncated at 290 amino acid residues by this mutation named *nup49-1*. Since *NUP49* was reported to be essential for viability, we replaced

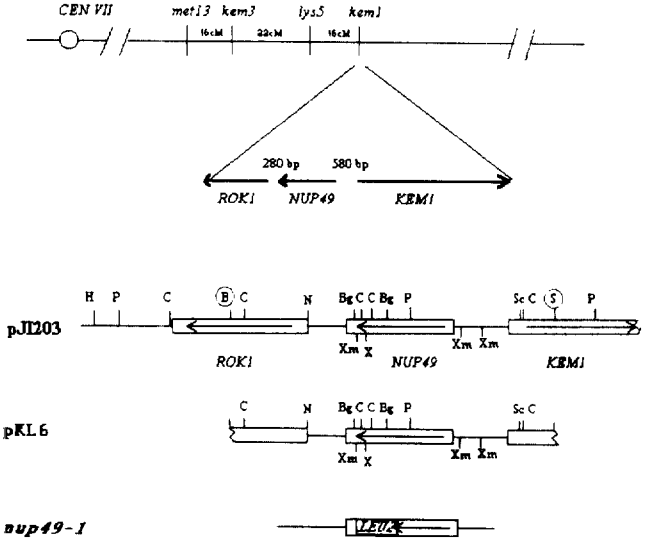

Fig. 1. Map location of *NUP49* on chromosome VII and the restriction maps of plasmid pJI203, pKL6, and *nup49-1* mutation. B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; H, *Hin*dIII; N, *Nco*I; P, *Pvu*II; S, *Sna*BI; Sc, *Sac*I.

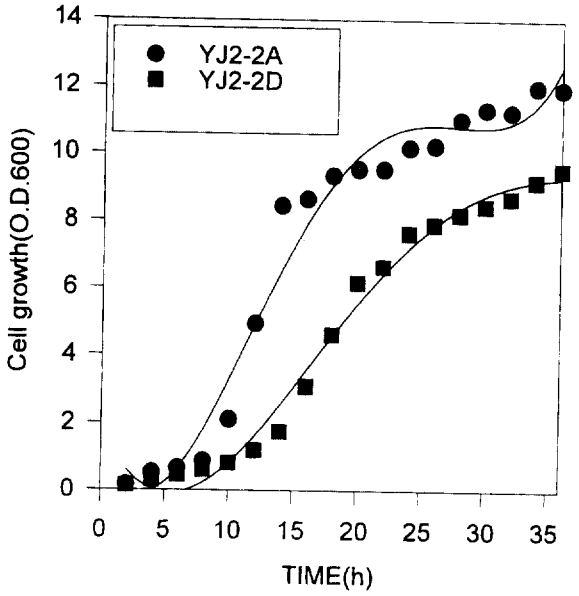

Fig. 2. Growth curve of YJ2-2A (*NUP49*) and YJ2-2D (*nup 49-1*) strains at 30°C. Optical density at 600 nm was measured at indicated time points.

one chromosomal copy of *NUP49* in a diploid strain F762/F763. The diploid transformant was sporulated and tetrads were analyzed. Despite the fact that deletion of the entire open reading frame of *NUP49* leads to lethality (16), deletion of one-third at the carboxy terminal end generates all four viable spores (data not shown). The growth curves of the wild type and the *nup49-1* mutant strains were analyzed and Fig. 2 shows the representative result with the sister spores, YJ2-2A (*NUP49*) and YJ2-2D (*nup49-1*). The mutant grows more slowly than the wild type. This result implies that the one-third of the carboxy-terminal of Nup49 (290~473 residues) may not be essential at normal growth temperature, although this part contains the Nsp1-like heptad repeats.

Conditional lethality of the *nup49-1* mutation

The growth patterns at various temperatures of the *nup49-1* mutant and the wild type strains were examined. As shown in Fig. 3, both strains grow at 30°C, whereas the *nup49-1* mutant showed temperature-sensitivity (ts) at 37°C and cold-sensitivity (cs) at 16°C. Both ts and cs were tested to be recessive (data not shown). These results suggest that the truncated Nup49 protein is still functional at the permissive temperature, but loses its primary functions or ability to interact with other proteins at nonpermissive temperatures. The Nup49 protein was reported to form a NPC subcomplex together with Nsp1 and Nic96 (7). We suspected that the carboxy-terminal region of Nup49 may be involved in protein-protein interactions, which was expected from its secondary structure analysis (18).

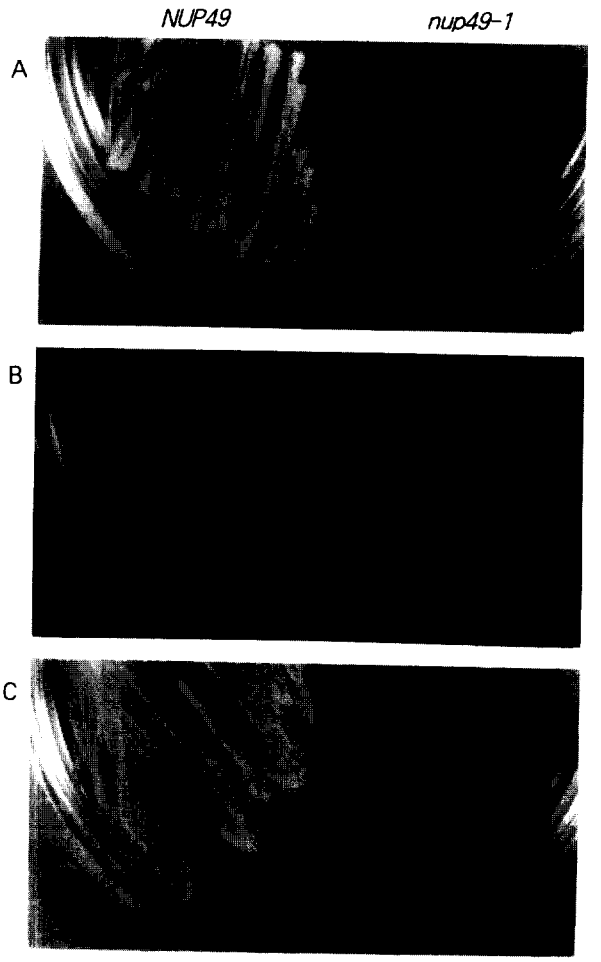

Fig. 3. Growth of wild type (*NUP49*) and *nup49-1* mutant strains at various temperatures. (A) 16°C, (B) 30°C, and (C) 37°C.

To ask whether or not *NUP49* functions at a specific stage of the cell cycle, the arrest phenotype of the *nup49-1* mutation was examined. Cultures grown at permissive temperature (30°C) were shifted to nonpermissive temperature (37°C) and cell morphology was quantitatively analyzed. As shown in Fig. 4, the arrested *nup49-1* mutants had the same distribution in cell morphology as the wild type, indicating a random arrest phenotype. Therefore, the Nup49 protein is expected to function throughout the cell cycle.

Since the null mutation in an essential gene results in lethality, any real study of the mutant phenotype is not available. Therefore, conditional lethality is very useful for the study of essential genes. This is especially the case for neucleoporins which are structural components without any easily detectable ezymatic activities. We expect that

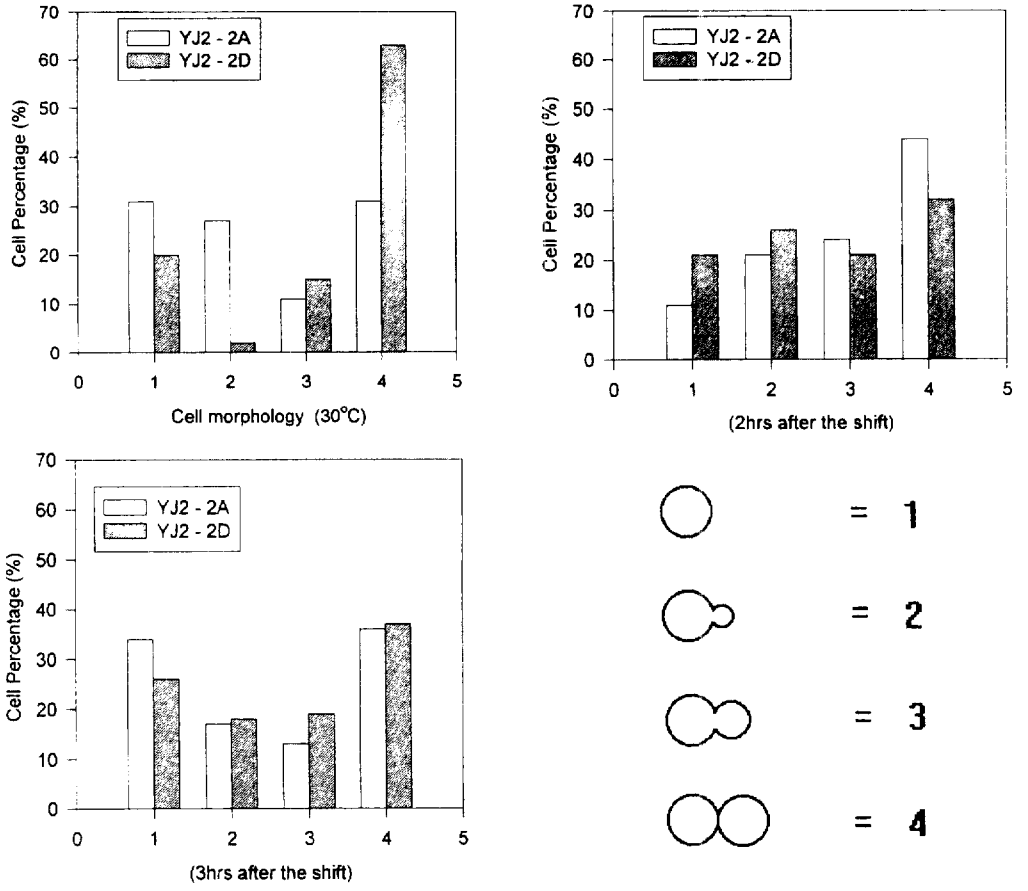

Fig. 4. Arrest phenotype of the *nup49-1* mutant strain after temperature shift to 37°C. (A) 30°C, (B) 2 h after the shift, (C) 3 h after the shift.

the phenotypic analysis of the *nup49-1* mutant may provide a good understanding of nucleotransport mechanisms and allow genetic approaches to identify new nucleoporin genes.

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