# NUP82 Is an Essential Yeast Nucleoporin Required for Poly(A)$^+$ RNA Export

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*Abstract.* We have isolated and characterized the gene encoding a novel essential nucleoporin of 82 kD, termed NUP82. Indirect immunofluorescence of cells containing an epitope tagged copy of the NUP82 localized it to the nuclear pore complex (NPC). Primary structure analysis indicates that the COOH-terminal 195 amino acids contain a putative coiled–coil domain. Deletion of the COOH-terminal 87 amino acids of this domain causes slower cell growth; deletion of the COOH-terminal 108 amino acids results in slower growth at 30°C and lethality at 37°C. Cells in which the last 108 amino acids of NUP82 have been deleted, when shifted to 37°C, do not display any gross morphological defects in their nuclear pore complexes or nuclear envelopes. They do, however, accumulate poly(A)$^+$ RNA in their nuclei at 37°C. We propose that NUP82 acts as a linker to tether nucleoporins directly involved in nuclear transport to pore scaffolding via its coiled–coil domain.

NUCLEAR pore complexes (NPCs)[1] serve as passageways for bidirectional transport of macromolecules between the nucleus and cytoplasm in all eukaryotes (for reviews see Rout and Wente, 1994; Fabre and Hurt, 1994). The NPC has an estimated mass of 125 megadaltons (MD) in vertebrates (Reichelt et al., 1990). Isolated yeast NPCs have an estimated mass of 60 MD and may contain up to 80 proteins (Rout and Blobel, 1993).

The genes encoding 11 yeast NPC proteins (termed NUP for nucleoporin) have been cloned. Most of these are members of two families defined by the repeated sequence motifs FXFG and GLFG. The FXFG family members include the proteins NUP1 (Davis and Fink, 1990), NUP2 (Loeb et al., 1993), NSP1 (Hurt, 1988; Nehrbass et al., 1990), and NUP159 (Gorsch et al., 1995; Kraemer et al., 1995). The GLFG family includes the yeast proteins NUP49 (Wente et al., 1992; Wimmer et al., 1992), NUP57 (Grandi et al., 1995), NUP100 (Wente et al., 1992), NUP116 (Wente et al., 1992; Wimmer et al., 1992), and NUP145 (Wente and Blobel, 1994; Fabre et al., 1994).

Recent studies indicate that repeat motif proteins from both families play a direct role in nuclear transport. The repeat domains of yeast and mammalian repeat proteins have been shown in overlay assays to interact with soluble import factors (Radu et al., 1995; Moroianu et al., 1995; Wu et al., 1995; Kraemer et al., 1995). However, protein transport may not be the only function of the peptide repeat containing proteins as evidenced by findings in yeast. Disruptions of the *NUP116* and *NUP145* genes result in striking pore and NE structural defects (Wente and Blobel, 1993, 1994) and a temperature sensitive mutant of NUP159 clusters its NPCs at permissive but not at restrictive temperature (Gorsch et al., 1995).

A structural phenotype has also been observed in NUP133, a nucleoporin that does not contain peptide repeats. Yeast with a disrupted *NUP133* gene cluster all their NPCs in a small region of the nuclear envelope (NE) (Doye et al., 1994; Pemberton et al., 1995; Li et al., 1995). These cells also have a defect in poly(A)$^+$ RNA export at restrictive temperature (Doye et al., 1994; Li et al., 1995), though no direct role in transport has been shown for NUP133. RNA export defects have been observed in yeast strains expressing mutants of other pore proteins, namely NUP49 (Doye et al., 1994), NUP116 (Wente and Blobel, 1993), NUP145 (Fabre et al., 1994), and NUP159 (Gorsch et al., 1995).

Aside from peptide repeats, coiled–coil domains, composed of α-helices wrapped around each other, are commonly found in yeast nucleoporins. Coiled–coils, which are detected by their characteristic spacing of hydrophobic residues in heptad repeats, mediate homotypic and heterotypic interactions in many different types of proteins (for review see Alber, 1992). Putative coiled–coils have been found in the peptide repeat containing nucleoporins NUP49, NUP57, NSP1, and NUP159 as well as in the nucleoporin NIC96 (Grandi et al., 1993), which does not con-

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1. *Abbreviations used in this paper*: HA, hemagglutinin; NE, nuclear envelope; NPC, nuclear pore complex; NUP, nucleoporin.

tain peptide repeats. Biochemical and genetic evidence have demonstrated that NUP49, NUP57, NSP1, and NIC96 can in part be isolated as a complex and it has been proposed that the coiled–coils mediate the binding of the various nucleoporins to each other (Grandi et al., 1995).

Here we report the identification and characterization of a novel essential nucleoporin, termed NUP82, with no homology to known nucleoporins. The COOH-terminal third of the protein contains a putative coiled–coil domain similar to that of other coiled–coil containing proteins. Deletion of part of the COOH-terminal coiled–coil enhances degradation of NUP82 and renders cells temperature sensitive and defective for poly(A)$^+$ RNA export at the restrictive temperature.

## Materials and Methods

### Fractionation of Yeast NPC Proteins

Enriched yeast NPCs were isolated from *Saccharomyces uvarum* as described by Rout and Blobel (1993), solubilized, and subjected to SDS-hydroxylapatite (SDS-HA) chromatography. An 82-kD protein was further separated from other polypeptides by reverse-phase HPLC of SDS-HA fractions (Wozniak et al., 1994). Aliquots of eluted fractions were prepared for and analyzed by SDS-PAGE as described (Radu et al., 1993).

Fractions that contain p82 were pooled, analyzed by SDS-PAGE and proteins were transferred to PVDF membranes and subjected to cleavage by endopeptidase Lys-C as described (Wozniak et al., 1994; Fernandez et al., 1992). Internal peptides were analyzed by $NH_2$-terminal sequencing (Fernandez et al., 1992).

### Isolation and Sequencing of the NUP82 Gene

The PCR procedure of Lee et al. (1988) was used to determine the cDNA sequence that encodes amino acid residues 419–424. Synthesis, subcloning and sequencing were as previously described (Radu et al., 1993) with the following modifications. Partially degenerate oligonucleotide primers corresponding to the sense strand of amino acid residues 413–418 and the antisense strand of amino acid residues 425–430 were synthesized and used with *Saccharomyces cerevisiae* DNA (Promega Biotech, Madison, WI) as a template in a PCR reaction. The reaction products were analyzed on a 10% polyacrylamide gel. A band of the expected size (53 bp) was excised, eluted and re-amplified under the same conditions, then excised, eluted, subcloned into pCR-Script (Stratagene Cloning Systems, La Jolla, CA), and sequenced with Sequenase Version 2.0 (USB Corp., Cleveland, OH).

A *S. cerevisiae* genomic library (Rose et al., 1989) was screened with the PCR product described above labeled with α[$^{32}$P]dATP using the Klenow fragment of DNA Polymerase I (New England Biolabs, Beverly, MA). Six filters, containing 10,000 colonies apiece, were screened exactly as described in Ausubel et al. (1994). Positive colonies were picked, plasmid DNA was purified and inserts were sequenced. All subcloning was done according to Sambrook et al. (1989).

The deduced amino acid sequence of the entire coding sequence of the protein was analyzed with Protean 1.08 from DNASTAR, Inc. (Madison, WI) and MacStripe 1.3.1 (Lupas, 1991; Knight, 1994) and was compared with the sequences in the Genbank and EMBL databases using the FASTA program of Pearson and Lipman (1988).

### Disruption of NUP82

A unique BglII site at position 666 of the coding sequence of *NUP82* was used to insert a BamHI fragment that encodes the *HIS3* gene. The resulting plasmid, pNUP82(HIS3), was linearized and used to transform diploid yeast (strain DF5, see Table I for details). Diploid transformants were sporulated and tetrads were dissected as described in Ausubel et al. (1994).

### Preparation of Yeast Extracts

DF5 or Y8 yeast were grown to mid-log phase in YPD or selective medium and then were pelleted, frozen in liquid nitrogen, thawed, solubilized in 1.85 M NaOH/7.5% β-mercaptoethanol and incubated on ice for 10 min. An equal amount of 50% trichloroacetic acid was added and incubated further on ice for 10 min. Protein aggregates were pelleted by centrifugation for 10 min in a microcentrifuge, washed with acetone and resuspended in sample buffer B (0.25 M Tris, 6.5% SDS, 0.1 M DTT, 12% glycerol, 0.008% Bromphenol blue). After sonication for 15 min in a bath sonicator, samples were incubated at 70°C for 15 min, and proteins were resolved by SDS-PAGE.

### Immunofluorescence of Yeast

Immunofluorescence was done as described in Wente et al. (1992) with the following modifications. Cells were fixed for 5 min in 0.1 M potassium phosphate, pH 6.5, 3.7% formaldehyde (Fluka Chemical Corp., Ronkonkoma, NY). After blocking, cells were incubated with the monoclonal antibody 12CA5 (Berkeley Antibody Co., Richmond, CA), which recognizes the epitope YPYDVPDYA from the influenza hemagglutinin (HA) protein, at a 1:2 dilution in buffer M (Wente et al., 1992) for 1 h at room temperature. Secondary antibody was Cy3-conjugated donkey anti–rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, PA) at a dilution of 1:50.

Epitope tagging was accomplished by ligating a PCR product of the endogenous promoter and coding sequence of *NUP82* into a plasmid (pJD305) which contains two tandem copies of the DNA encoding the HA epitope fused in frame at the 3′ end of the gene ( plasmid pNUP82-2μ; see Table II for more details).

### Immunoblot Analysis

Proteins were separated by SDS-PAGE and transferred onto nitrocellulose. The blots were washed in $H_2O$, blocked in TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl), 5% non-fat dry milk (Nestle Food Co., Glendale, CA), and then incubated with the 12CA5 monoclonal antibody at a dilution of 1:100 in TBS/1% milk for 90 min. Blots were washed four times in TBS and then incubated with goat anti–mouse antibody conjugated to horseradish peroxidase (1:1,000) (Amersham Corp., Arlington Heights, IL) in TBS/1% milk for 30 min and washed as before. Antibody–antigen

*Table I. Yeast strain genotype and construction*

| Strain | Genotype | Derivation |
|---|---|---|
| DF5 | Mat**a**/Matα his3-Δ200/his3-Δ200 leu2-3,112/leu2-3,112 lys2-801/lys2-801 trp1-1/trp1-1 ura3-52/ura3-52 | Finley et al. (1987) |
| DF5α | Matα his3-Δ200 leu2-3,112 lys2-801 trp1-1 ura3-52 | |
| Y8 | Mat**a**/Matα his3-Δ200/his3-Δ200 leu2-3,112/leu2-3,112 lys2-801/lys2-801 trp1-1/trp1-1 ura3-52/ura3-52 nup82::HIS3/+ | Integrative transformation of DF5 with linearized pNUP82(HIS) |
| NUP82-wt | Matα his3-Δ200 leu2-3,112 lys2-801 trp1-1 ura3-52 nup82::HIS3 pNUP82-wt(LEU) | Transformation of Y8 with pNUP82-wt, sporulation, Leu$^+$/His$^+$ segregant |
| NUP82-Δ87 | Matα his3-Δ200 leu2-3,112 lys2-801 trp1-1 ura3-52 nup82::HIS3 pNUP82-Δ87(LEU) | Transformation of Y8 with pNUP82-Δ87 sporulation, Leu$^+$/His$^+$ segregant |
| NUP82-Δ108 | Matα his3-Δ200 leu2-3,112 lys2-801 trp1-1 ura3-52 nup82::HIS3 pNUP82-Δ108(LEU) | Transformation of Y8 with pNUP82-Δ108, sporulation, Leu$^+$/His$^+$ segregant |

complexes were visualized using the ECL system as described by the manufacturer (Amersham Corp.). Kodak XAR film was used (Eastman Kodak Co., Rochester, NY).

### *In Vivo Poly(A)$^+$ RNA Accumulation Assay*

Yeast strains NUP82-wt, NUP82-Δ87 and NUP82-Δ108 (see Table I for details) were grown overnight in YPD at 30°C. Cultures were diluted 1:10 in YPD, grown for another 2 h, and then diluted 1:1 with YPD and incubated for 3 h at either 30° or 37°C. The RNA accumulation assay was performed as described in Wente and Blobel (1993) (see also Forrester et al., 1992). The probe used was digoxigenin labeled $(dT)_{30}$ at a concentration of 20 ng/ml. FITC conjugated anti-digoxigenin antibody (Boehringer Mannheim Corp., Indianapolis, IN) was used at a 1:4 dilution to visualize RNA–DNA hybrids.

## *Results*

### *Isolation of NUP82 and Cloning of Its Gene*

Previously, a procedure was developed to isolate yeast NPCs (Rout and Blobel, 1993). An ~80-kD protein coenriched with NPCs and was further purified by SDS-hydroxylapatite chromatography and reverse phase HPLC (Wozniak et al., 1994). Peptides from the 80-kD protein were sequenced and one of them, a 21–amino acid peptide, was used to make a DNA probe for screening a yeast genomic plasmid library.

A ~9-kb clone was isolated which contains an open reading frame encoding a protein of 713 amino acids with a predicted mass of 82 kD (Fig. 1 *A*). Because this protein is indeed a nucleoporin (see below), it was termed NUP82. NUP82 contains all the peptide sequence derived from the 80-kD protein (Fig. 1 *A*). Analysis of the primary structure of NUP82 with the program MacStripe 1.3.1 predicts that the COOH-terminal 197 amino acids contain heptad repeats and form a coiled–coil domain (Fig. 1 *B*). A search of the Genbank and EMBL databases showed significant homology between the COOH-terminal 195 amino acids of NUP82 and other coiled–coil protein domains (14–27% identity, 41–52% similarity) including those of myosins. The $NH_2$-terminal 518–amino acid region shows no similarity to any protein in the Genbank or EMBL databases.

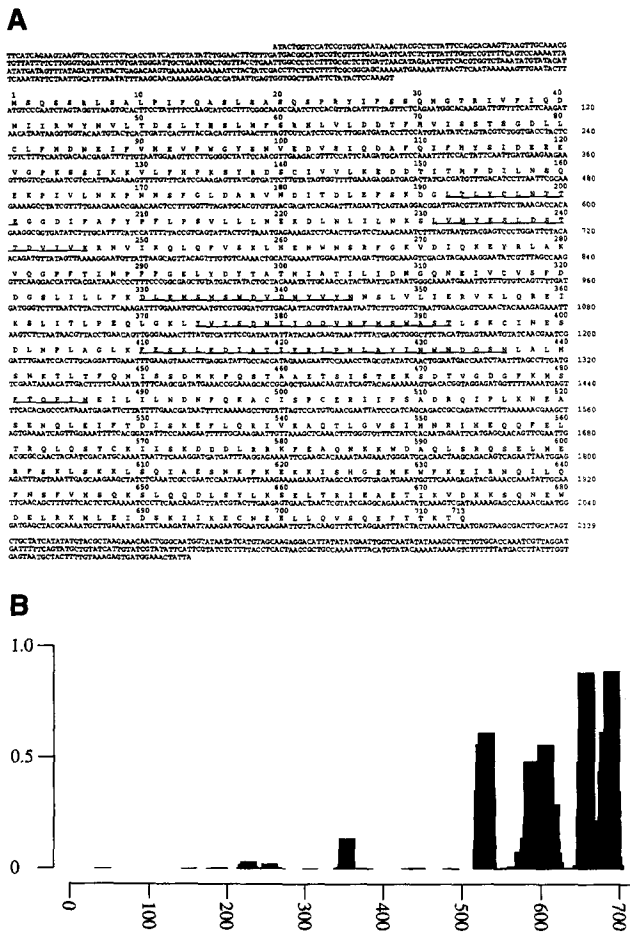


*Figure 1.* (*A*) Nucleotide and predicted amino acid sequence of the *NUP82* locus. Nucleotides are numbered on the right. Amino acid residues, represented by their one letter code, are numbered over the sequence beginning with the initiator methionine. Sequences obtained by peptide sequencing are underlined. (*B*) Plot of predicted coiled coil region from MacStripe 1.3.1 (a 21–amino acid window was used). The horizontal axis numbers represent residues in the NUP82 sequence. The vertical axis numbers represent the probability of coiled–coil formation.

*Table II. Plasmid construction*

| Plasmids | Construction |
|---|---|
| pNUP82 | 4.5-BamHI kb BamHI/HindIII fragment that contains the *NUP82* locus in BamHI/HindIII of pBluescript SK$^+$ |
| pNUP82(HIS) | BamHI fragment that contains *HIS3* gene from YDpH in unique BglIII site of pNUP82 |
| pRS316-82 | The BamHI/HindIII fragment that contains the *NUP82* locus inserted into pRS316 |
| pJD305 | A modification of YEplac181 that contains the following cassette between two HindIII sites: a 5′ SphI site, then ~4 kb of DNA followed by two copies of the DNA that encodes an epitope of the influenza hemagglutinin protein with a 5′ in frame KpnI site and a 3′ stop codon and the cyc1 terminator sequence |
| pNUP82-2μ | A PCR product of the *NUP82* locus and 510 bp of 5′ DNA preceded by an SphI site and followed by an in frame 3′ KpnI site in place of a stop codon inserted into the unique SphI and KpnI sites of pJD305 |
| pNUP82-wt | The HindIII fragment from p82-2μ that contains the *NUP82* locus inserted into pRS315 |
| pNUP82-Δ87 | Identical to p82-2μ except the PCR product is missing the last 261 nucleotides of *NUP82* |
| pNUP82-Δ108 | Identical to p82-2μ except the PCR product is missing the last 324 nucleotides of *NUP82* |

References for pRS315 and pRS316 (Sikorski and Heiter, 1989); Yeplac181 (Geitz and Sugino, 1988); pJD305 (Dohmen et al., 1995).

### *NUP82 Is Essential for Viability*

Disruption of *NUP82* with the *HIS3* gene was carried out as described in Fig. 2 *A*. Insertion of the *HIS3* gene allows the cells to survive on medium lacking histidine. Sporulation and dissection of the heterozygous diploid strain (Y8) resulted in two or fewer viable spores per tetrad (Fig. 2 *B*), implying that the *NUP82* is essential for viability. All viable spores were unable to survive on medium lacking histidine, indicating that they do not contain the disruption. To confirm that *NUP82* is essential, Y8 yeast were transformed with pRS316-82, a single copy plasmid containing the *URA3* gene and the *NUP82* gene under its endogenous promoter. After transformants were sporulated and dissected, spores were grown on plates lacking histidine to select for yeast with a disruption in *NUP82*. Colonies were then streaked onto plates containing 5-fluoro-orotic acid (5-FOA), which is toxic to cells that harbor the *URA3* gene. None of the yeast survived on 5-FOA plates (data not shown), and therefore could not survive after the loss of the plasmid, demonstrating that *NUP82* is essential for growth.

### *Immunolocalization of NUP82*

To determine the location of NUP82 in the cell, indirect immunofluorescence was performed on a strain that contains an epitope-tagged version of NUP82, termed NUP82-wt. Two copies of the DNA encoding a nine amino acid sequence from the influenza HA protein were inserted in frame at the 3′ end of the *NUP82* gene in the single copy plasmid pRS315, which contains the *LEU2* gene, to make pNUP82-wt. Y8 yeast were transformed with pNUP82-wt, sporulated and dissected. A $Leu^+/His^+$ haploid strain was recovered (NUP82-wt), indicating that an epitope tagged NUP82 can functionally replace the wild type protein. These cells were prepared for indirect immunofluorescence and probed with the monoclonal antibody 12CA5, which recognizes the HA epitope. Punctate staining of the nuclear rim, a characteristic of nucleoporins, was observed (Fig. 3). Given its staining pattern and the purification of

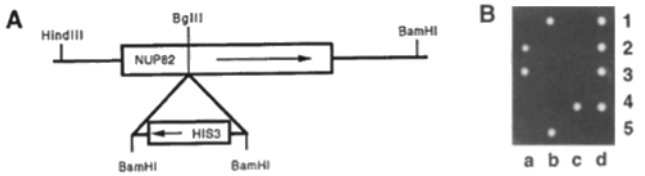


*Figure 2. NUP82* is required for cell viability. The *NUP82* gene in the plasmid Bluescript $SK^+$ was disrupted by inserting DNA that encodes the *HIS3* gene in a unique BglII site at position 666 of the *NUP82* open reading frame shown in *A*. The plasmid was linearized with HindIII and integrative transformation into DF5 diploid yeast was performed. The diploid strain was sporulated and tetrads dissected as shown in *B*. Numbers refer to tetrads, letters to spores within a tetrad.

NUP82 from a fraction enriched in NPC proteins, we conclude that NUP82 is a nucleoporin.

### *The COOH Terminus Is Required for Stability of NUP82*

To investigate the role of the COOH-terminal coiled–coil domain of NUP82, we expressed mutants of NUP82 which are missing parts of the 197 amino acid COOH-terminal coiled–coil in a *NUP82* null strain. Two partial deletions of *NUP82* were constructed and inserted into the multicopy plasmid pJD305; in one, 261 nucleotides were removed from the 3′ end (corresponding to 87 amino acids) and in the other 324 nucleotides were removed (corresponding to 108 amino acids). Both of these constructs were epitope tagged at their 3′ ends exactly as was done for wild type *NUP82*. The resulting proteins are called NUP82-Δ87 and NUP82-Δ108, respectively (Fig. 4 *A*).

At 30°C, *NUP82* null strains transformed with either of these constructs (strains NUP82-Δ87 and NUP82-Δ108) survived but grew slightly more slowly than the same strain transformed with pNUP82-wt (NUP82-wt), which contains the full length *NUP82* gene (Fig. 4 *B*, *left*). However, 3 h after the shift to 37°C, NUP82-Δ108 cells grew significantly more slowly than at 30°C and stopped grow-

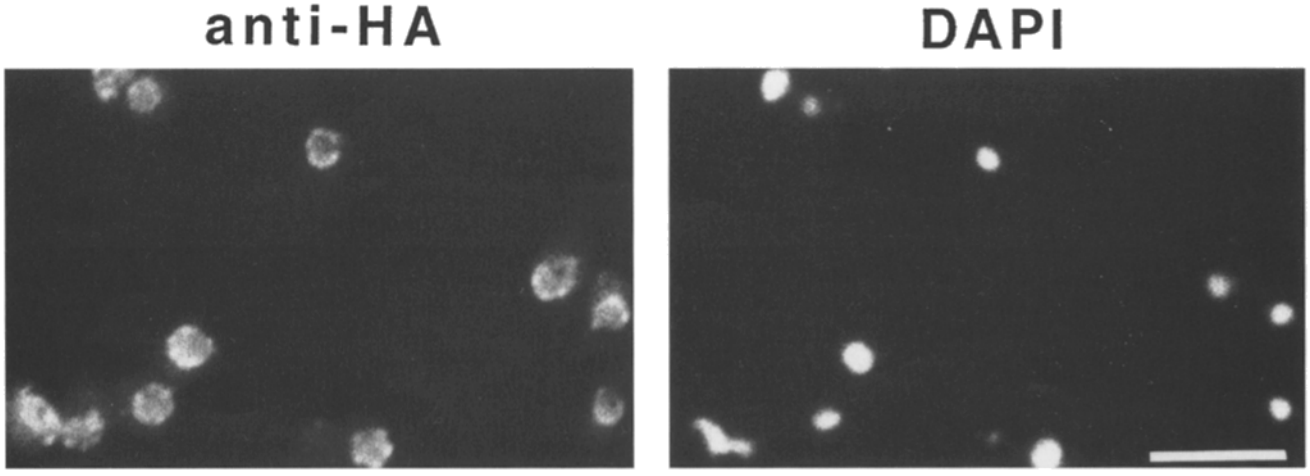


*Figure 3.* Immunolocalization of an epitope tagged NUP82 by indirect immunofluorescence microscopy. Cells from the haploid yeast strain NUP82-wt (nup82::HIS3 transformed with pNUP82-wt) were prepared for immunofluorescence and then probed with anti-HA (12CA5) antibody followed by Cy3-conjugated donkey anti-mouse antibody (*left*). Coincident staining of the same cells with DAPI is shown on the right. Bar, 5 μm.

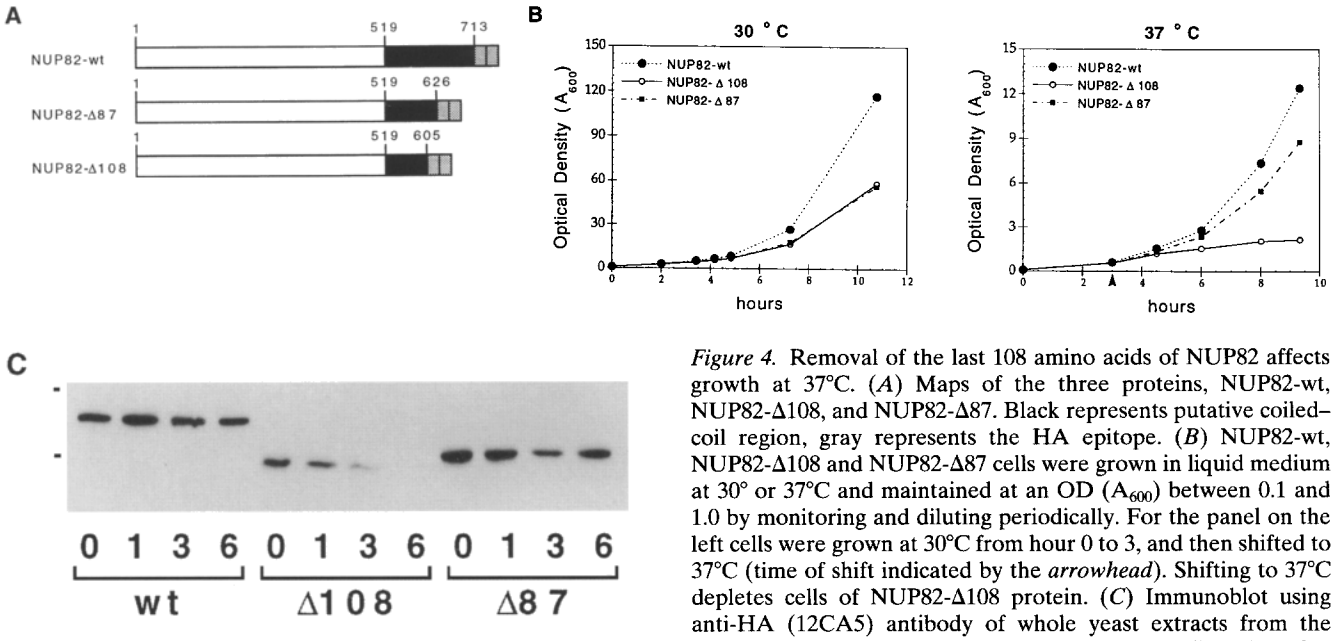


*Figure 4.* Removal of the last 108 amino acids of NUP82 affects growth at 37°C. (*A*) Maps of the three proteins, NUP82-wt, NUP82-Δ108, and NUP82-Δ87. Black represents putative coiled–coil region, gray represents the HA epitope. (*B*) NUP82-wt, NUP82-Δ108 and NUP82-Δ87 cells were grown in liquid medium at 30° or 37°C and maintained at an OD ($A_{600}$) between 0.1 and 1.0 by monitoring and diluting periodically. For the panel on the left cells were grown at 30°C from hour 0 to 3, and then shifted to 37°C (time of shift indicated by the *arrowhead*). Shifting to 37°C depletes cells of NUP82-Δ108 protein. (*C*) Immunoblot using anti-HA (12CA5) antibody of whole yeast extracts from the three yeast strains growing at 30°C (0 time point) and at 1, 3, and 6 h after shift to 37°C. Equivalent amounts of cells (based on OD [$A_{600}$]) were loaded onto each lane. Bars represent 97 and 66 kD. The lower level of protein observed in the 3 h lane of NUP82-Δ87 is due to loading error.

ing after 6 hours; both NUP82-wt and NUP82-Δ87 cells grew slightly slower than they did at 30°C (Fig. 4 *B*, *right*).

To determine the steady state levels of the three epitope tagged proteins, immunoblot analysis using the anti-HA monoclonal antibody was performed on the three strains grown at 30°C, and then at 37°C for 1, 3, and 6 h. Fig. 4 *C* shows that the levels of NUP82-wt and NUP82-Δ87 are the same at both temperatures, but the amount of NUP82-Δ108 significantly decreases soon after the temperature shift and is almost undetectable after 6 h. Even at 30°C the steady state level of NUP82-Δ108 is lower than that of the other two proteins despite that all three genes share the same promoter and therefore presumably have the same level of transcription. These data indicate that the last 108 amino acids of NUP82 are required for the stability of the protein as increased degradation of NUP82-Δ108 is seen at the restrictive temperature.

### *Depletion of NUP82 Has No Detectable Effect on Morphology*

The ability to gradually deplete cells of NUP82-Δ108 by shifting them to 37°C allowed us to test whether this was accompanied by changes in NPC or nuclear envelope morphology. However, no gross morphological defects were detected. Thus, NUP82-Δ108 depleted cells yielded the characteristic punctate nuclear rim shown in indirect immunofluorescence using the monoclonal antibody 414 (data not shown), which recognizes nucleoporins (Davis and Blobel, 1986). Indirect immunofluorescence using 12CA5 antibody shows punctate nuclear rim staining in all NUP82-Δ108 cells grown at 30°C. As expected, the staining becomes weaker as the time of growth at 37°C is increased (at 6 h only background staining is seen), consistent with the depletion of the protein after the temperature shift (data not shown). Moreover, examination of NUP82-Δ108 cells grown at 30°C and then at 37°C (for 6 h) by thin section electron microscopy did not show alterations in NPC distribution or nuclear envelope structure.

### *NUP82-Δ108 Yeast Are Defective for Poly(A)$^+$ RNA Export at Restrictive Temperature*

The lack of gross morphological changes led us to search for a phenotype based on NPC function to account for the lethality seen on temperature shift in the NUP82-Δ108 yeast strain. Cells were tested for poly(A)$^+$ RNA accumulation in the nucleus by hybridization with digoxigenin labeled $(dT)_{30}$ and visualized with FITC-conjugated anti–digoxigenin antibody. Fig. 5 shows that poly(A)$^+$ RNA accumulates in the nuclei of NUP82-Δ108 cells at the restrictive temperature (*lower right panel*) but not at the permissive temperature (*upper right panel*). NUP82-wt (Fig. 5, *left*) and NUP82-Δ87 (data not shown) cells did not accumulate RNA in their nuclei at either temperature.

An in vivo nuclear import assay using the reporter protein NLS-β-galactosidase (Underwood and Fried, 1989) was performed on the strains described above. Because expression of the reporter was extremely low in NUP82-Δ108 cells, probably due to the mRNA export defect, we were unable to conclude whether NUP82-Δ108 cells were competent for nuclear import at the restrictive temperature (see Discussion).

## *Discussion*

We have isolated and characterized a novel yeast nucleoporin, NUP82, that is essential for cell growth. At the primary structural level NUP82 has no homology with any known proteins. NUP82 has a putative coiled–coil domain at its COOH terminus, a motif it shares with four other yeast nucleoporins: NUP49, NUP57, NSP1, and NIC96,

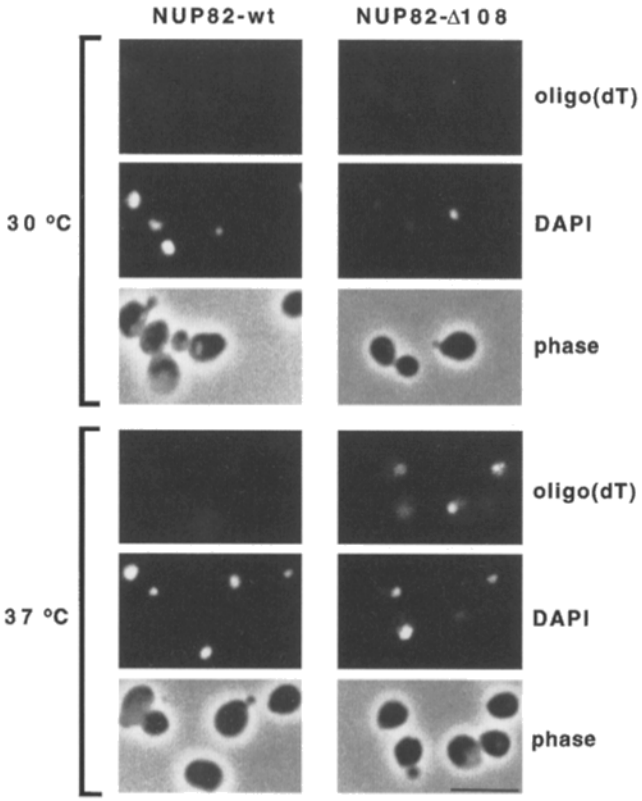


*Figure 5.* Poly(A)$^+$ RNA accumulates in the nuclei of NUP82-Δ108 cells at restrictive temperature. Cells from the haploid yeast strains NUP82-wt and NUP82-Δ108 were prepared for in situ hybridization and then probed with digoxigenin labeled poly(dT)$_{30}$ followed by FITC-conjugated anti-digoxigenin antibody (at top). Left panel shows cells grown at 30°C. Right panel shows cells grown at 37°C for 3 h. Coincident DAPI staining and phase contrast images are shown underneath each photograph. All photographs were taken and printed with the same exposure times. Bar, 5 μm.

which form a subcomplex of the NPC (Grandi et al., 1993, 1995). Since coiled–coil domains interact with one another, any of these proteins are candidates for interaction with NUP82. It is also possible that NUP82 dimerizes or forms multimers by its coiled–coil as has been shown in vitro for the vertebrate nucleoporin p62 (Buss et al., 1994).

Analysis of the coiled–coil domain of NUP82 showed that the deletion of the last 108–amino acid residues confers temperature sensitivity on NUP82-Δ108 cells. This effect is most likely due to destabilization of the protein and subsequent degradation, indicated by the decrease in protein level to nondetectable amounts after 6 h at 37°C. Notably, a slightly smaller deletion of the COOH terminus (87 amino acids) did not result in decreased levels of protein at 37°C. Nonetheless, NUP82-Δ87 cells grow more slowly than NUP82-wt cells at all temperatures, indicating that removal of the COOH-terminal 87 amino acids does affect the function of the NUP82 protein. This defect, however, is not severe enough to cause temperature sensitivity or nuclear RNA accumulation. Whether the effect is caused by misfolding of NUP82-Δ87 or by the loss of an interaction mediated by that segment of the coiled–coil domain remains to be determined.

We have shown that depletion of NUP82 results in an RNA export defect. Taking into account the structural features of NUP82 (i.e., the lack of repeat motifs and the presence of a coiled–coil domain) several possible scenarios for NUP82 function can be considered. NUP82 may interact directly with factors involved in RNA export. Mutations in other nucleoporins result in export defects but in contrast to most of these nucleoporins, NUP82 mutant cells show no morphological abnormalities. The NPCs from NUP116 mutant cells are sealed over by the NE, resulting in an export defect independent of NPC function (Wente and Blobel, 1993). Similarly, cells containing a mutant NUP145 have grape-like clusters of NPCs in their NEs (Wente and Blobel, 1994) which might also account for their inability to export RNA (Fabre et al., 1994). Yeast lacking NUP133 also display an export defect (Doye et al., 1994; Li et al., 1995) and abnormal localization of NPCs in the NE (Doye et al., 1994; Pemberton et al., 1995; Li et al., 1995) though these two effects may be separate. The NE and NPC morphology of NUP49 mutant cells, the other protein whose mutation causes RNA accumulation in the nucleus (Doye et al., 1994), has not been studied.

Alternatively, NUP82 may be necessary for nuclear import of RNA processing factors, without which RNA cannot be exported from the nucleus. An in vivo nuclear import assay did not show any mislocalization of a reporter protein to the cytoplasm but its expression level was very low in NUP82-Δ108 cells at the restrictive temperature. This result is consistent with an inability of NUP82-Δ108 cells to make the reporter protein at the restrictive temperature due to its inability to export mRNA. NUP82-Δ108 cells may have impaired nuclear import but if so, it is obscured by and could be secondary to the severe poly(A)$^+$ RNA export defect.

In a third model, NUP82 plays a structural role in the NPC. The lack of a morphological phenotype for NUP82-Δ108 at the restrictive temperature demonstrates that NUP82 is unlikely to be involved in the maintenance of NE structure or pore location in the NE. However, NUP82 may play a more subtle structural role which, when compromised, might abolish pore function completely but cause no gross morphological changes in the NPC. Such a defect could account for the poly(A)$^+$ RNA accumulation seen at restrictive temperature in NUP82-Δ108 cells.

In such a scenario, NUP82 plays an intermediate role between core proteins which, when deleted cause striking pore and NE abnormalities, and the nucleoporins on the NPC surface which interact directly with soluble transport factors. NIC96 may be in the same class as NUP82. It, too, has a coiled–coil which, when partially truncated, confers temperature sensitivity on the cell and displays transport defects (Grandi et al., 1995). However, morphological studies have not been performed on NIC96 mutants; it may be different from NUP82 in that respect.

To date, studies of the nucleoporins have concentrated on those which contain peptide repeats. Now, a second, overlapping, family of nucleoporins is emerging, those which contain coiled–coil domains. Though not unique to nucleoporins, the coiled–coil domain may be one of the major structural motifs through which nucleoporins bind to each other.

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