A Novel Nuclear Pore Protein Nup82p Which Specifically Binds to a Fraction of Nsp1p

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Abstract. Nsp1p interacts with nuclear pore proteins Nup49p, Nup57p and Nic96p in a stable complex which participates in nucleocytoplasmic transport. An additional p80 component is associated with Nsp1p, but does not co-purify with tagged Nup57p, Nup49p and Nic96p. The p80 gene was cloned and encodes a novel essential nuclear pore protein named Nup82p. Immunoprecipitation of tagged Nup82p reveals that it is physically associated with a fraction of Nsp1p which is distinct from Nsp1p found in a complex with Nup57p, Nic96p and Nup49p. The Nup82 protein can be divided into at least two different domains both required for the essential function, but it is only the carboxy-terminal domain, exhibiting heptad repeats, which binds to Nsp1p. Yeast cells depleted of Nup82p stop cell growth and concomitantly show a defect in poly(A)$^+$ RNA export, but no major alterations of nuclear envelope structure and nuclear pore density are seen by EM. This shows that Nsp1p participates in multiple interactions at the NPC and thus has the capability to physically interact with different NPC structures.

THE major structural features of the nuclear pore complex have now been firmly established. Each nuclear pore complex (NPC)[1] is composed of the spoke complex sandwiched by an outer (cytoplasmic) and inner (nucleoplasmic) ring and the central plug (transporter). Attached to the inner ring is the nuclear basket and protruding from the outer ring are eight short filaments and particles (for review see Fabre and Hurt, 1994; Panté and Aebi, 1994; Rout and Wente, 1994). Some of these structures must be directly involved in bi-directional nucleocytoplasmic transport, which occurs exclusively through the nuclear pore complexes (Feldherr et al., 1984; Richardson et al., 1988). Concerning active nuclear import of proteins, which is NLS mediated and energy dependent, it was suggested that the central plug could serve as a transporter (Akey, 1992), whereas the channels between the spokes (Hinshaw et al., 1992) or between the transporter and the spokes (Akey and Radermacher, 1993) may allow passive diffusion of small molecules; furthermore, the short cytoplasmic filaments and the nuclear baskets were proposed to be involved in the early docking steps at the NPC for protein import and RNA export, respectively (Feldherr et al., 1984; Richardson et al., 1988; Mehlin et al., 1992).

Whereas a mechanistic picture for the cytoplasmic phase of nuclear protein import is beginning to emerge (Powers and Forbes, 1994), very little is known about the following steps at the NPC, in particular which nuclear pore proteins are involved in the actual translocation process and how they act. It is likely that some of the cytosolic/nuclear factors required for nucleocytoplasmic transport such as the NLS receptor complex initially identified by Adam and Gerace (1991) and later shown to be importin 60/90 (Görlich et al., 1994; Görlich et al., 1995) which is also called karyopherin α/β (Moroianu et al., 1995; Radu et al., 1995), Hsc70 (for review, see Goldfarb, 1992), the small GTPase Ran/TC4 (Moore and Blobel, 1994*b*), its guanine nucleotide exchanger RCC1 (Kadowaki et al., 1993; Tachibana et al., 1994), and the Ran-associated protein B2 (Moore and Blobel, 1994*a*) may transiently interact with NPC components during transport of the substrate through the NPC channel, but how this occurs is still unknown. The observation that substrates for nuclear import can bind to the nuclear envelope under conditions in which translocation is inhibited was taken as evidence that NLS receptor(s) can dock at the NPC presumably by binding to specific NPC proteins. Nuclear protein import is inhibited by microinjection of the lectin wheat germ agglutinin (WGA) that binds to a family of NPC proteins (Finlay et al., 1987; Dabauvalle et al., 1990). The interaction of this family of nuclear pore proteins with cytosolic transport factors is also

The sequence data for the *NUP82* gene are available from Genbank/EMBL/DDBJ under accession number X85970.

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1. *Abbreviations used in this paper*: NPC, nuclear pore complex; nt, nucleotide; TFA, trifluoroacetic acid; WGA, wheat germ agglutinin.

suggested by the fact that immobilized rat liver NPC proteins on WGA-Sepharose resin could deplete a soluble activity from the cytosolic extract required for nuclear transport in permeabilized mammalian cells (Sterne-Marr et al., 1992). Importin 60/90 (Görlich et al., 1994; Görlich et al., 1995) or karyopherin α/β (Moroianu et al., 1995; Radu et al., 1995) are sufficient to mediate the nuclear pore binding of the karyophile and therefore represent the cytosolic NLS-receptor; interestingly, importin 60/karyopherin α show sequence homology to yeast Srp1p (Yano et al., 1992) which is localized at the NPC and physically associated with bona fide nucleoporins such as Nup1p and Nup2p (Belanger et al., 1994). Accordingly, Srp1p is a good candidate for a nuclear import factor which (transiently) associates with components of the nuclear pore complex. Another interesting example of a physico-functional interaction of soluble transport factors and nuclear pore proteins is the recent finding that nucleoporins such as Nup2p have homology to Ran-binding proteins (Dingwall, C., personal communication) and thus could interact with Ran/TC4, another key factor in nucleocytoplasmic transport (Moore and Blobel, 1994*b*).

There is evidence suggesting a role of NPC proteins in both nuclear protein import and RNA export. Antibodies against nucleoporins as well as WGA were used to inhibit nuclear accumulation of proteins and RNA export reactions (Featherstone et al., 1988; Finlay et al., 1987). Immunodepletion of nuclear pore proteins such as p62 and its associated p58 and p54 components from *Xenopus* egg extracts used to reconstitute nuclei caused inhibition of nuclear protein uptake in this in vitro system (Finlay et al., 1991). In the yeast system, nuclear pore proteins were shown to be required for in vivo accumulation of nuclear reporter proteins inside the nucleus by analyzing conditionally lethal nucleoporin mutants at the restrictive condition. Among the tested NPC proteins, Nsp1p (Mutvei et al., 1992; Nehrbass et al., 1993), Nup49p (Doye et al., 1994), Nic96p (Grandi et al., 1995) and Nup1p (Bogerd et al., 1994) exhibited defects in uptake of nuclear reporter proteins. Nucleoporin mutants were also analyzed for an impaired mRNA export by in situ hybridization (Amberg et al., 1992; Kadowaki et al., 1992) and Nup116p (Wente and Blobel, 1993), Nup145p (Fabre et al., 1994), Nup49p (Doye et al., 1994), Nup133p (Doye et al., 1994; Li et al., 1995), Nup1p (Bogerd et al., 1994; Schlaich and Hurt, 1995), and Nup159p (Gorsch et al., 1995) are involved in the export reaction. Clearly, some of the nuclear pore proteins, such as Nup1p, are required for both efficient nuclear protein import and RNA export. Interestingly, different mutant alleles of a single nucleoporin, as shown for Nup49p, can specifically affect nuclear protein accumulation or poly(A)$^+$ RNA export (Doye et al., 1994).

Because of these allele-specific effects on nucleocytoplasmic transport, it is important to analyze with which protein(s) a single nucleoporin physically interacts. This may help to elucidate the mechanism by which NPC proteins are connected to different nucleocytoplasmic transport machineries. Subcomplexes of the nuclear pore complex have been described from both higher eukaryotes and yeast. Examples are the p62 complex isolated from rat liver nuclei or *Xenopus* egg extracts and composed of three subunits p62, p58, and p54 (Finlay et al., 1991; Dabauvalle et al., 1990; Kita et al., 1993; Buss and Stewart, 1995) and a hetero-dimeric complex from rat consisting of glycoproteins p250 and p75 (Panté et al., 1994). In yeast, immunoprecipitation studies using anti-Srp1p antibodies revealed that Srp1p, which was initially identified as a suppressor of RNA polymerase I mutants (Yano et al., 1992), is associated with Nup1p and Nup2p nucleoporins (Belanger et al., 1994). On the other hand, Nup1p and Nup2p do not stably associate with each other. Since *NUP1* and *NUP2* interact genetically (Loeb et al., 1993), the most likely explanation for this data is functional redundancy between the Nup1p-Srp1p and Nup2p-Srp1p complexes. We have previously shown that nucleoporin Nsp1p, the yeast homologue of p62 (Carmo-Fonseca et al., 1991), is physically associated with several proteins including nucleoporin Nup49p, Nup57p, and Nic96p (Grandi et al., 1993). Furthermore, a core complex is built up by Nsp1p, Nup49p, and Nup57p, whereas Nic96p is more loosely associated via its heptad repeats containing domain (Grandi et al., 1995). Functional interactions in vivo can be inferred by the fact that combinations of mutant alleles among these four nucleoporins cause synthetic lethal phenotypes (Grandi et al., 1995).

In the course of purifying Protein A-tagged Nsp1p by IgG-Sepharose chromatography, besides Nic96p, Nup57p, and Nup49p, also a p80 component co-purified (Grandi et al., 1993). However, when other tagged members of the Nsp1 complex were affinity-purified, this p80 component was absent. To further investigate this specific interaction between Nsp1p and p80, we cloned the p80 gene. Here, we show that Nup82p (corresponding to p80) is a novel nucleoporin which interacts with a fraction of Nsp1p and has a role in poly(A)$^+$ RNA transport.

Materials and Methods

Yeast Strains, Media, and Microbiological Techniques

In Table I, the yeast strains used in this study are listed. Yeast cells were grown on minimal SD/SGAL medium supplemented with all amino acids and nutrients except those used for the selection (CSM medium; BIO101, La Jolla, CA) or rich YPD/YPGal medium; plasmid transformation, gene disruption, sporulation of diploid cells, and tetrad analysis were performed essentially as described in Wimmer et al. (1992).

Plasmids

The following yeast plasmids were used in this study: pUN100, *ARS/CEN* plasmid with the *LEU2* marker (Elledge and Davis, 1988); pSB32, *ARS/CEN* plasmid with the *LEU2* marker (Nehrbass et al., 1990); *pRS316*, *ARS/CEN* plasmid with the *URA3* marker (Sikorski and Hieter, 1989); pRS314, *ARS/CEN* plasmid with the *TRP1* marker (Sikorski and Hieter, 1989); YEp13-Matα2-lacZ (Nehrbass et al., 1993), 2 μ high copy number plasmid with the *LEU2* marker; pCH1122-NSP1 (Wimmer et al., 1992), *ARS/CEN* plasmid with the *URA3* marker.

Purification of Nup82p and Peptide Sequencing

For the generation of the internal peptide sequences derived from the p80 component, the purified ProtA-Nsp1p complex was analyzed by SDS-12% polyacrylamide gel electrophoresis and the Coomassie blue stained p80 band (see Fig. 1) was excised from the Laemmli slab gel (amberlite MB-6-treated acrylamide/bisacrylamide). The p80 band was digested in the gel matrix with trypsin (1–2 mg of TPCK-treated trypsin) according to Eckerskorn and Lottspeich (1989). The digestion was performed at 37°C for 20 h, and the derived peptides were eluted from the gel with 10% trifluoroacetic acid (TFA) in water. Peptides were separated by reverse

Table I. Yeast Strains

Strain	Genotype
RS453	*a/α, ade2/ade2, trp1/trp1, leu2/leu2, ura3/ura3, his3/his3*
PG2	*a/α, ade2/ade2, trp1/trp1, leu2/leu2, ura3/ura3, his3/HIS3::nup82/NUP82*
NUP82 shuffle	*a, ade2, trp1, leu2, ura3, HIS3::nup82,* (pRS316-URA3-NUP82); haploid progeny derived from PG2
ProtA-Nup82	*a, ade2, trp1, leu2, ura3, his3, HIS3::nup82,* (pUN100-LEU2-ProtA-Nup82)
GAL::ProtA-NUP82	*a, ade2, trp1, leu2, ura3, his3, HIS3::nup82,* (pUN100-GAL10::ProtA-NUP82)
NUP82$^+$	*a, ade2, trp1, leu2, his3, NUP82*; haploid progeny derived from RS453
nup133$^-$	*a, ade2, trp1, leu2, his3, ura3, nup133::HIS3* (Doye et al., 1994)
Ala6-nsp1	*a, ade2, trp1, leu2, his3, ura3, Ala6-nsp1::HIS3* (Wimmer et al., 1993)
Ala6-nsp1/ProtA-Nup82Δ624-713	*α, ade2, trp1, leu2, ura3, Ala6-nsp1::HIS3, nup82::HIS3,* (pCH1122-URA3-NSP1), (pUN100-LEU2-NUP82Δ624-713)

phase HPLC using a C_{18} column (Vydac, 4.6 × 250 mm) with a Waters 600 HPLC (Millipore). The solvent system used was 0.1% (vol/vol) TFA in water (aqueous phase) and 0.085% TFA in acetonitrile (organic phase). After an initial lag phase of 10 min at 2% organic phase, a gradient of 2–30% organic phase was run over 63 min (1% per 2.25 min) using a flow rate of 0.5 ml/min. Fractions were collected manually according to the absorption at 215 nm monitored by a Waters model 486-UV detector. Peptides were identified by Edman sequencing and mass spectrometry. Automated sequence analysis of the purified peptides was performed using a type 473A protein sequencer (Applied Biosystems). Electrospray ionisation mass spectroscopy of the peptides was performed using a Finnigan TSQ MAT 700 mass spectrometer.

Cloning, Sequencing, and Disruption of the NUP82 Gene

The four peptide sequences obtained from the sequence analysis of the p80 band were all retained within an ORF of a yeast genomic sequence as part of chromosome X. Cosmid 28 which contains this ORF is 40,311-bp long and inserted into vector pWE15 (Huang et al., 1994). The position of *NUP82* ORF in the cosmid 28 is 2,406–4,547 bp. The MIPS code for *NUP82* is p1 B713 and the working nomenclature is J1135. The *NUP82* gene was excised as a 3.9-kb long HindIII/SnabI restriction fragment from cosmid 28 and subcloned into pUN100 previously cut with HindIII and SmaI. A SacI/HindIII restriction fragment containing the entire *NUP82* gene was excised from the construct pUN100-NUP82 and cloned also into pRS316 vector generating pRS316-NUP82. To disrupt the *NUP82* gene, the *HIS3* gene isolated as a 1.1-kb-long BamHI and blunt-ended fragment was inserted into the EcoRV site thereby interrupting the Nup82p ORF at amino acid 204 (Asp). The *nup82::HIS3* gene was excised from pBluescript as a BamHI/SacI fragment and used to transform the diploid strain RS453. HIS3$^+$ transformants which contained the *nup82::HIS3* integration at the *NUP82* gene locus were verified by Southern analysis and the heterozygous strain PG2 was sporulated and tetrad analysis was performed.

Construction of ProtA-NUP82, ProtA-nup82Δ624-713, ProtA-nup82Δ460-713, ProtA-nup82(499-713), and GAL::ProtA-NUP82

To tag Nup82p, two IgG binding units from *S. aureus* protein A were joined to the NH_2-terminal end of Nup82p. For this gene fusion, a new *Sac*I restriction site was first generated after the ATG codon of *NUP82* at position 410 by PCR-mediated mutagenesis (**GAG CTC** TCC CAA TCT AGT AGG TTA AGT) and a HindIII site at position 2758 in the 3′ non-coding sequence of *NUP82*. A SacI/HindIII restriction fragment corresponding to the *NUP82* gene (but lacking the ATG start codon) was ligated in the correct orientation to a 1.1-kb SacI/SacI restriction fragment corresponding to two IgG binding units plus the *NOP1* promoter (derived from pUN100-ProtA-NIC96 (Grandi et al., 1993)), and the whole fusion gene was inserted as SacI/HindIII fragment in pUN100.

The same *ProtA-NUP82* fusion gene under the control of the *NOP1* promoter was also inserted as a BamHI/HindIII fragment into pRS314 generating pRS314-ProtA-NUP82.

To construct ProtA-Nup82pΔ624-713 (which lacks about 1/2 of the carboxy-terminal heptad repeat domain), pUN100-ProtA-NUP82 was cut at the unique NcoI site (nt 2277, amino acid 624 [Ser]), blunt-ended and an oligonucleotide containing stop codons in all three reading frames (Grandi et al., 1995) was inserted.

To construct the ProtA-nup82Δ460-713, the *ProtA-Nup82pΔ624-713* fusion gene with the *NOP1* promoter was excised from pUN100-ProtA-Nup82 as a BamHI/HindIII fragment and cloned in pSB32 vector. Here, the oligonucleotide containing stop codons in all the three reading frames (Grandi et al., 1995) was inserted at a unique PvuII site in the sequence of *NUP82* ([nucleotide] nt 1748, amino acid 459[Ala]).

To tag the heptad repeat containing domain of Nup82p, a SacI site was generated at nt 1900 (amino acid 499[Cys]) in the sequence of *NUP82* by PCR (**GAG CTC** CTG TAT TAG TCC ATG) as well as a HindIII site at nt 2758 using the same primer used to construct the *ProtA-NUP82* fusion gene. The PCR product was cut with SacI and HindIII restriction enzymes and joined in frame with the 1.1-kb SacI/SacI fragment containing two IgG-binding units and the *NOP1* promoter in pUN100 opened at its SacI/HindIII sites thereby generating the fusion gene *NOP1* promoter-*ProtA-nup82(499-713)*.

To construct the *GAL10::ProtA-NUP82* fusion gene, a SacI/SacI fragment containing the *URA3-GAL10::ProtA* DNA was isolated from pBluescript-URA3-GAL10::ProtA-NIC96 (Grandi et al., 1993) and inserted into the SacI site of pUN100-ProtA-NUP82 thereby replacing the *NOP1* promoter and *ProtA* DNA sequences. Thus, a *ProtA-NUP82* fusion gene under the control of the *GAL10* promoter was generated. Finally, the *URA3* gene in the plasmid was inactivated by inserting an XbaI

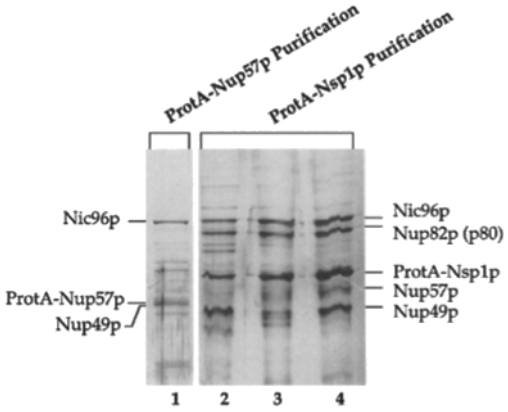

Figure 1. Nup82p (p80) co-purifies with ProtA-Nsp1p, but does not associate with ProtA-Nup57p. Extraction of ProtA-Nsp1p and ProtA-Nup57p with various lysis buffers and affinity-purification by IgG-Sepharose chromatography are described under Materials and Methods. A 300-fold equivalent of the purified fractions (ProtA-Nup57p Purification or ProtA-Nsp1p Purification) eluted from the affinity columns by acidic pH and analyzed by SDS-8% acrylamide gel electrophoresis and silver staining is shown. The positions of the nucleoporin bands are indicated on both sides of the gels. Note that (*a*) the band corresponding to full-length Nsp1p in lane *1* is not visible due to Nsp1p proteolysis as verified by Western blotting decorated with anti-Nsp1 antibodies (see also Fig. 4 *B*); and (*b*) purified ProtA-Nup57p and ProtA-Nsp1p were not analysed on the same gel but were aligned according to the position of Nic96p. The following lysis buffers were used: (lanes *1* and *3*), 20 mM NaCl, 2% Triton X-100, 20 mM Tris-HCl, pH 8.0; (lane *2*) 100 mM NaCl, 0.2% desoxycholate, 0.02% SDS, 1% NP-40, 50 mM Tris-HCl, pH 8.0; (lane *4*) 150 mM KCl, 20 mM Tris-HCl, pH 8.0.

linker (1010; Biolabs) into the ApaI site (located within *URA3*). pUN100-GAL10::ProtA-NUP82 was transformed into PG2 and LEU$^+$ transformants were selected. After tetrad analysis on YPGal-plates, haploid progeny was isolated which were *nup82::HIS3* and carried pUN100-GAL10::ProtA-NUP82. These cells were checked for the galactose-dependent expression of the ProtA-Nup82p fusion protein on Western blots as described (Grandi et al., 1993). Repression of transcription of the fusion gene and the subsequent growth phenotype was measured by shifting the GAL::ProtA-NUP82 strain from galactose to glucose containing medium (YPGal and YPD).

Construction of Synthetic Lethal Strains

In order to combine the *Ala6-nsp1* allele with the *ProtA-Nup82pΔ624-713* mutant, strain Ala6-nsp1 containing pCH1122-URA3-NSP1, and the NUP82 shuffle strain, complemented by the pUN100-ProtA-NUP82Δ624-713 were mated. Diploids growing on SD-ura-leu selective medium were sporulated. Haploid progeny was selected which was HIS$^+$, URA$^+$, and LEU$^+$. One of these progeny, Ala6-nsp1/ProtA-NUP82Δ24-713, was plated on 5-FOA-containing plates to induce the loss of the pCH1122-URA3-NSP1 plasmid. The Ala6-nsp1/ProtA-NUP82Δ624-713 strain was also transformed with the TRP1-containing pRS314-ProtA-NUP82 plasmid before growth on 5-FOA-plates.

Immunoprecipitation and Indirect Immunofluorescence

Immunoprecipitation experiments were performed as described in Bergès et al. (1994) with the following modifications: the lysis buffer used was 0.2% Triton X-100, 150 mM KCl, 20 mM Tris-HCl, pH 8, 5 mM $MgCl_2$ and a protease inhibitor cocktail; the antibodies used were anti-Nsp1 rabbit immune serum (EC10-2) (Hurt, 1988), anti-Nup57 rabbit immune serum, anti-GLFG rabbit immune serum (Grandi et al., 1995), rabbit anti–chicken IgG (Medac, Hamburg, Germany). Note that all these specific IgG rabbit antibodies cross-react with the ProtA-tag of the used fusion proteins on Western blots due to the general binding of Protein A to the constant region of rabbit IgG molecules. The strains used for the immunoprecipitation experiments were grown in YPD medium. If strains expressing non-functional *nup82* mutant alleles were used they were grown in selective SDC medium. Strains were grown at 30°C prior to immunoprecipitation. Immunofluorescence experiments to analyse nuclear protein import and mRNA export phenotypes were performed as described (Doye et al., 1994). As nuclear reporter the fusion protein Matα2-lacZ was used (Doye et al., 1994). Since the *LEU2* marker in the nup82 mutant strains was no longer available, the pRS316-URA3-Matα2-lacZ was constructed. Accordingly, a SalI/HindIII restriction fragment containing the *Matα2-lacZ* fusion gene was cut from YEp13-LEU2-Matα2-lacZ and cloned into pRS316-URA3.

Thin Section Electron Microscopy

Thin section EM which was performed essentially according to Byers and Goetsch (1991) is described in Doye et al. (1994). Briefly, GAL::ProtA-nup82 cells grown for 9 h in glucose-containing medium were fixed in 2% paraformaldehyde/2% glutaraldehyde before the cell wall was removed by glusulase (Dupont; NEN, Boston, MA) and zymolyase 20T (Seikagaku Corp., Tokyo, Japan). Postfixation was done in 2% osmium tetroxyde followed by en block staining in 1% uranyl acetate. Dehydrated samples were embedded in Epon according to standard procedures. Samples were contrasted by staining with uranyl acetate and Renold's lead.

Miscellaneous

DNA manipulations (restriction analysis, end filling reaction, ligation, PCR amplification, etc.) were done essentially according to Maniatis et al. (1982). Isolation of total yeast DNA and Southern analysis was done as described in Sherman, 1990.

Results

Nup82p Specifically Co-purifies with ProtA-Nsp1p

When Nsp1p tagged with IgG-binding sequences derived from protein A was affinity-purified under non-denaturing conditions, several prominent bands co-isolated. Among these bands Nup49p, Nup57p, and Nic96p were shown to form a complex with Nsp1p and also to functionally interact as revealed by genetic analysis (Grandi et al., 1993, 1995). The eluate from the IgG-Sepharose column, however, contained a band of approximately 80 kD (termed p80) whose intensity somehow varied from preparation to preparation, but could be recovered under different extraction and purification conditions (Fig. 1, *ProtA-Nsp1p purification*). Initially, this band was considered to be less specific, because p80, in contrast to Nic96p, could still associate with the Ala6-nsp1p mutant in which 6 charged residues were changed to neutral alanines within the Nsp1p carboxy-terminal domain (Wimmer et al., 1993). Surprisingly, when ProtA-Nup57p (Fig. 1, *ProtA-Nup57p purification*), ProtA-Nup49p (Grandi et al., 1993), or ProtA-Nic96p (data not shown) were affinity purified under the same conditions, all the other members of the complex co-purified, but p80 was absent. This suggested that a fraction of Nsp1p interacts with p80 whereas another pool of Nsp1p molecules is organized in a complex with Nup49p, Nup57p, and Nic96p.

To further characterize the separate Nsp1p-containing complexes, we sought to clone the gene encoding the p80 component. The p80 band, corresponding to about 15–20 μg of protein was excised from SDS–polyacrylamide slab gels and sequence data were obtained for four tryptic peptides (Fig. 2). Two peptides were long enough to generate degenerate oligonucleotide primers to clone the p80 gene by PCR from a yeast genomic library. At the same time, a cosmid clone of about 40 kb in length containing part of yeast chromosome X was sequenced (Pohl, T., GATC, Konstanz, Germany). At one end of the insert, a long open reading frame was found which contained the four tryptic peptides derived from the p80 component (Fig. 2). As deduced from the DNA sequence, this open reading frame is capable of encoding a protein of 713 amino acids or 82-kD molecular weight which fits well with the apparent molecular mass of p80 on SDS-PAGE (Figs. *1* and *2*). The gene was therefore designated *NUP82* (for Nuclear Pore Protein of 82 kD, see also later). The Nup82p amino acid sequence does not reveal significant homology to known protein sequences present in the data libraries nor does it contain FSFG and GLFG repeat sequences as found in other NPC proteins. However, the last 200–amino acid residues of Nup82p are predicted to form a coiled–coil structure due to the presence of heptad repeats (Fig. 2, *lower panel*). Similar heptad repeat sequences are also present in several other nucleoporins including Nsp1p, Nup49p, Nup57p, and Nic96p (Wimmer et al., 1992; Grandi et al., 1993, 1995).

NUP82 Gene Disruption, Localization, and Purification of ProtA-Nup82p

To investigate whether the *NUP82* gene is essential for cell growth, we performed either a gene disruption or repression of gene expression. For the gene disruption, the *HIS3* marker was inserted at a convenient restriction site of the *NUP82* gene thereby interrupting its open reading frame (see Materials and Methods). After transformation and selection for HIS$^+$ transformants, heterozygous diploids *HIS3::nup82/NUP82* were obtained by homologous

Amino Acid Sequence of Nup82p

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  1  MSQSSRLSAL PIFQASLSAS QSPRYIFSSQ NGTRIVFIQD NIIRWYNVLT
 51  DSLYHSLNFS RHLVLDDTFH VISSTSGDLL CLFNDNEIFV MEVPWGYSNV
101  EDVSIQDAFQ IFHYSIDEEE VGPKSSIKKV LFHPKSYRDS CIVVLKEDDT
151  ITMFDILNSQ EKPIVLNKPN NSFGLDARVN DITDLEFSKD GLTLYCLNTT
201  EGGDIFAFYP FLPSVLLLNE KDLNLILNKS LVMYESLDST TDVIVKRNVI
251  KQLQFVSKLH ENWNSRFGKV DIQKEYRLAK VQGPFTINPF PGELYDYTAT
301  NIATILIDNG QNEIVCVSFD DGSLILLFKD LEMSMSWDVD NYVYNNSLVL
351  IERVKLQREI KSLITLPEQL GKLYVISDNI IQQVNFMSWA STLSKCINES
401  DLNPLAGLKF ESKLEDIATI ERIPNLAYIN WNDQSNLALM SNKTLTFQNI
451  SSDMKPQSTA AETSISTEKS DTVGDGFKMS FTQPINEILI LNDNFQKACI
501  SPCERIIPSA DRQIPLKNEA SENQLEIFTD ISKEFLQRIV KAQTLGVSIH
551  NRIHEQQFEL TRQLQSTCKI ISKDDDLRRK FEAQNKKWDA QLSRQSELME
601  RFSKLSKKLS QIAESNKFKE KKISHGEMKW FKEIRNQILQ FNSFVHSQKS
651  LQQDLSYLKS ELTRIEAETI KVDKKSQNEW DELRKMLEID SKIIKECNEE
701  LLQVSQEFTT KTQ*
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PEPCOIL-Analysis of Nup82p

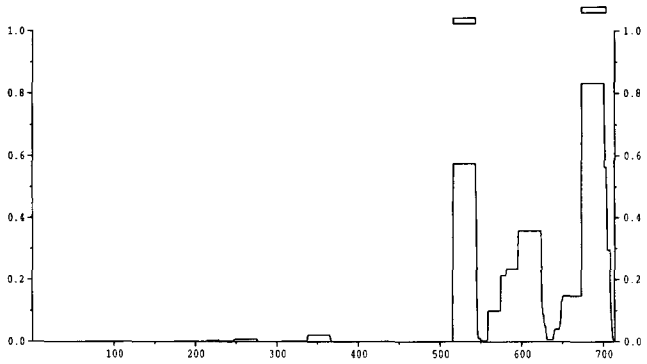

Figure 2. Amino acid sequence of Nup82p. (*A*) Predicted amino acid sequence of the Nup82p protein as deduced from the DNA sequence. Underlined are the four peptide sequences obtained from the amino acid sequence analysis of the p80 band and found within the Nup82p ORF. (*B*) Prediction of coiled–coil regions in the Nup82p carboxy-terminal domain; the program PEPCOIL was applied which reveals potential coiled–coil regions in protein sequences using the algorithm of Lupas et al. (1991).

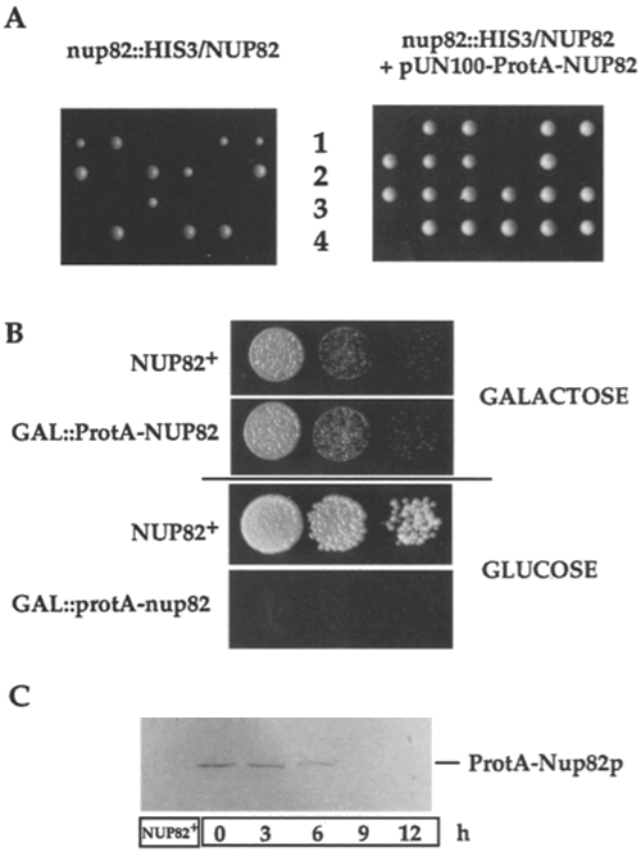

Figure 3. Disruption of the *Nup82* gene or repression of *NUP82* gene expression causes cell death. (*A*) Tetrad analysis of diploid strain PG2 which is heterozygous for *NUP82* (*nup82::HIS3/NUP82*) and PG2 transformed with pUN100-ProtA-NUP82. (*B*) Growth of strains GAL::Prot-NUP82 and isogenic NUP82$^+$ on galactose-containing (YPGal) and glucose-containing (YPD) plates. Equivalent amount of cells and derived 1/10 and 1/100 dilutions were spotted onto the indicated plates and incubated at 30°C for 3 d. By this "dot spotting," the relative growth rates of the used strains can be directly scored on plates. (*C*) Immunoblot analysis of cell extracts derived from NUP82$^+$ and GAL10::ProtA-NUP82 strains after the indicated times in glucose-containing YPD medium. The same amounts of cell equivalents from a whole cell extract were analysed by SDS-PAGE and Western blotting. The ProtA-Nup82p fusion protein was visualized using IgG coupled to HRP.

recombination. Sporulation of heterozygous integrants and tetrad analysis gave a 2:2 segregation for viability (Fig. 3 *A*), and the his$^-$ genotype co-segregated with the growing haploid progeny. Microscopic inspection of germinated *HIS3::nup82* progeny showed that cells stopped growth at the two to four cell stage. The lethal phenotype of the *HIS3::nup82* disruption mutant can be complemented by the cloned *NUP82* gene present on a single copy plasmid (Fig. 3 *A*).

To repress *NUP82* gene expression, the authentic promoter of *NUP82* was replaced by the *GAL10* promoter; in addition, the Nup82p was tagged with IgG-binding sequences derived from *Staphylococcus aureus* protein A at its amino terminal end in order to follow the Nup82p depletion during gene repression. A haploid GAL::ProtA-NUP82 strain was constructed (see Materials and Methods) which grew normally in galactose-containing medium, but completely stopped cell growth after about 12 h shift in glucose-containing medium as compared to an isogenic NUP82$^+$ strain (Fig. 3 *B*). Concomitant with gene repression, ProtA-Nup82p disappeared from cell extracts (Fig. 3 *C*).

To determine the subcellular localization of Nup82p, we tagged it with protein A. The ProtA-Nup82p fusion protein is functional and complements the lethal *nup82::HIS3* mutant (Fig. 3 *A*). As expected, by indirect immunofluorescence with anti-ProtA antibody, a ring-like and punctate staining of the nuclear envelope is observed which is similar to the immunofluorescence staining of other yeast nucleoporins (Fig. 4 *A*, *upper panel*). In addition, when expressed in the nup133$^-$ strain which shows clustered NPCs, ProtA-Nup82p also clustered together with other nucleoporins (Fig. 4 *A*, *lower panel*). This nup133 mutant allows to test whether a given protein is physically linked to the nuclear pore complexes (Doye et al., 1994). This data shows that Nup82p is localized at the NPC and sug-

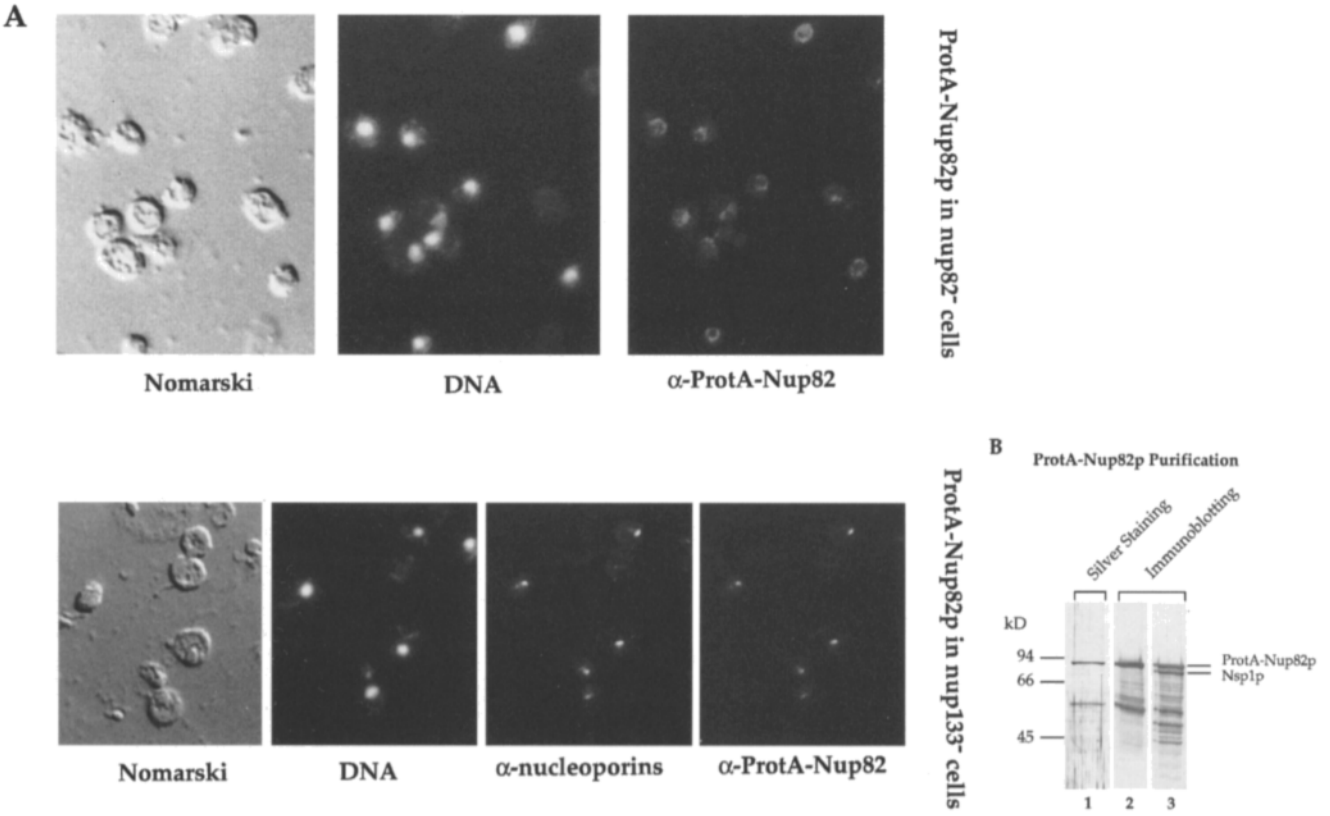

Figure 4. Intracellular location and purification of ProtA-Nup82p. (*A*) Indirect immunofluorescence was performed on strains nup82⁻ and nup133⁻, both expressing ProtA-Nup82p. To detect ProtA-Nup82p, rabbit anti-chicken IgG was used as first antibody followed by goat anti-rabbit IgG coupled to Texas red. A ring-like and punctate staining of the nuclear periphery is observed in nup82⁻ cells, whereas clustering together with other nucleoporins is seen in nup133⁻ cells. To detect yeast nucleoporins, mAb414 (BAbCO, Berkley, CA) was used. Cells were also stained for DNA using Hoechst 33258 and viewed by Nomarski optics. (*B*) Affinity-purification of ProtA-Nup82p was done as described under Materials and Methods. The eluate from the IgG-Sepharose column was analyzed by SDS-PAGE and Silver staining (lane *1*) and immunoblotting (lanes *2* and *3*) using IgG coupled to HRP to visualize ProtA-Nup82p fusion protein (lane *2*) and anti-Nsp1 antibodies to detect Nsp1p (lane *3*). The positions of ProtA-Nup82p, Nsp1p and a molecular weight standard are indicated. Note that in (lane *1*) the faint band migrating slightly faster than ProtA-Nup82p corresponds to full-length Nsp1p and that anti-Nsp1 antibodies also recognize the ProtA-Nup82p fusion protein. The strong band around 50 kD is a major breakdown product of ProtA-Nup82p since it is also detected by Western blotting using IgG-HRP.

gests that it is not an unspecific contaminant of affinity-purified Nsp1p.

ProtA-Nup82p, expressed in the *nup82* disrupted strain, was also affinity-purified under non-denaturing conditions by IgG-Sepharose chromatography (Fig. 4 *B*). Following the same extraction and purification protocol as applied for ProtA-Nsp1p (Grandi et al., 1993), microgram quantities of the tagged ProtA-Nup82p fusion protein (as derived from about 10^8 yeast cells) could be obtained in a one-step purification. As revealed by SDS-PAGE and Silver staining, ProtA-Nup82p mainly purifies as a single band of about 90-kD apparent molecular weight, but several other weaker stained bands became also visible (Fig. 4 *B*). Major co-purifying bands (e.g., Nic96p) were not seen. When the purified ProtA-Nup82p fraction was analyzed by Western blotting using different anti-nucleoporin antibodies, Nic96p, Nup57p, and Nup49p were not present (see also later), but Nsp1p could be clearly detected (Fig. 4 *B*, lane *3*). This Nsp1p band corresponds to one of the substoichiometric bands in the silver-stained preparation; since Nsp1p is very susceptible to proteolysis, it was partly degraded during ProtA-Nup82p purification (compare also Fig. 4 *B*, lanes *2* and *3*, *Immunoblotting*).

A Fraction of Nsp1p, Free of Nup49p, Nup57p, and Nic96p, Is Specifically Associated with ProtA-Nup82p via Heptad Repeats Interactions

To confirm the existence of two pools of Nsp1p associated with either Nup82p or the Nic96p/Nup57p/Nup49p-containing nucleoporin complex, native immunoprecipitation of ProtA-Nup82p and Nup57p was performed successively from a whole cell lysate (Fig. 5 *A*). The immune pellets and supernatants were then analyzed by Western blotting and probed for the presence of ProtA-Nup82p, Nsp1p, Nup49p, Nup57p, and Nic96p. As seen in Fig. 5 *B*, ProtA-Nup82p can be immunoprecipitated with an efficiency of about 15–20% using rabbit IgGs (P 1, α-ProtA-Nup82). If probed with anti-Nsp1 antibodies, Nsp1p is also found in this immune pellet, whereas Nup57p, Nup49p, and Nic96p are completely absent. The remaining supernatant S1 was split in half and subjected to a second round of immunoprecipita-

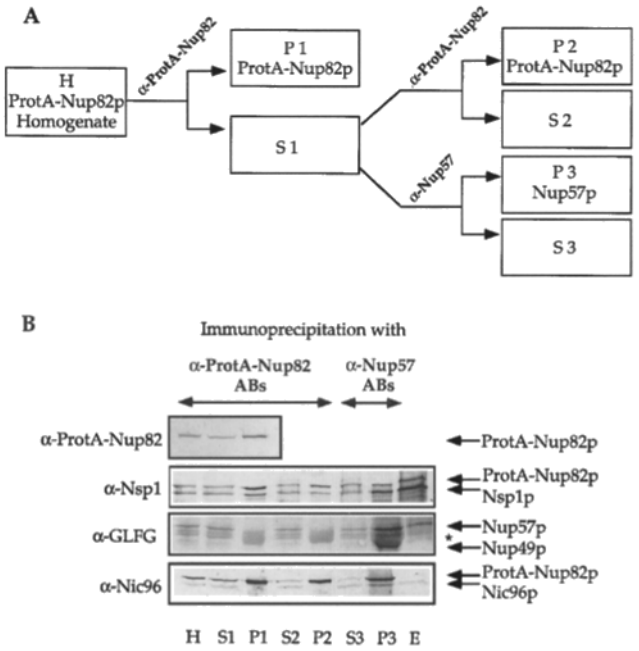

Figure 5. Native immunoprecipitation of ProtA-Nup82p. (*A*) Flow chart of the native immunoprecipitation starting with a cell extract derived from a yeast strain expressing ProtA-Nup82p. *H*, homogenate; *P*, immune pellet; *S*, immune supernatant. (*B*) Immunoprecipitation from ProtA-Nup82 cell lysates under non-denaturing conditions as described under Materials and Methods using rabbit IgGs to immunoprecipitate the protein A moiety of ProtA-Nup82p (*α-ProtA-Nup82 ABs*). The derived immune supernatant was split and one half was re-incubated with rabbit IgGs to re-immunoprecipitate ProtA-Nup82p (*α-ProtA-Nup82 ABs*), whereas the other half was immunoprecipitated using anti-Nup57 monospecific antibodies (*α-Nup57 ABs*). A onefold equivalent of the homogenate (*H*) and immune supernatant (*S*) and a five-fold equivalent of the immune pellet (*P*) were analyzed by SDS-PAGE before blotting onto nitrocellulose. (*E*) Whole cell lysate of a wild-type RS453 strain. Blots were then probed with IgG coupled to HRP, anti-Nsp1p (*α-Nsp1*), anti-GLFG (*α-GLFG*; reactive with both *Nup57p* and *Nup49p*), and anti-Nic96 (*α-Nic96*) antibodies to detect ProtA-Nup82p, Nsp1p, Nup49p, Nup57p, and Nic96p, respectively. The co-precipitating bands are indicated by arrows, the star marks IgG heavy chain. Note that all the specific antibodies also recognize the ProtA-Nup82 fusion protein (see also under Materials and Methods), because of the ProtA-moiety which binds to any rabbit IgG. This is especially evident with anti-Nsp1 and anti-Nic96 antibodies. For the co-migration of Nup2p and ProtA-Nup82p bands, see legend to Fig. 6 *B*.

tion, using either anti-ProtA-Nup82 reagents as described above or monospecific anti-Nup57 antibodies. Nsp1p, but not Nup49p, Nup57p, and Nic96p, still co-precipitated with ProtA-Nup82p (P 2, α-ProtA-Nup82), whereas anti-Nup57 antibodies brought down Nic96p, Nup57p, Nup49p plus a larger portion of Nsp1p (Fig. 5 *B*, *P3*, α-Nup57). These data show that Nup82p is specifically associated with a separate fraction of Nsp1p, whereas another pool of Nsp1p is in a complex with Nup49p, Nup57p, and Nic96p.

Nup82p exhibits heptad repeats in its carboxy-terminal domain which give a probability of coiled–coil secondary structure (Fig. 2). As previously shown, heptad repeats in Nsp1p and Nic96p are responsible for the physical association with the other nucleoporins of the complex (Grandi et al., 1995). In order to see whether the heptad repeat domain in Nup82p is involved in the physical interaction with Nsp1p, the carboxy-terminal domain of Nup82p alone tagged with ProtA was expressed in yeast. Although ProtA-Nup82p(499-713) did not complement the nup82$^-$ strain, it still could bind to Nsp1p as shown by immunoprecipitation (Fig. 6 *A*). This binding was specific, since ProtA-Nup82p(499-713) was not at all associated with Nup57p (Fig. 6 *A*). Furthermore, a carboxy-terminally truncated Nup82p mutant tagged with protein A was generated, Nup82pΔ460-713, which lacks all of the heptad repeats (see also Fig. 2) and can not complement the nup82$^-$ strain (data not shown). As shown by native immunoprecipitation, deletion of the carboxy-terminal domain of Nup82p impairs the binding to Nsp1p (Fig. 6 *B*).

To demonstrate that Nsp1p and Nup82p also functionally interact via their carboxy-terminal domains in the living cell, haploid yeast progeny was constructed containing the functional protein A tagged Nup82pΔ624-713 construct which lacks about half of the heptad repeats and, accordingly, binds less efficiently to Nsp1p (data not shown), and the thermo-sensitive Ala6-nsp1p allele which was integrated at the *NSP1* locus and thus replaced the wild-type *NSP1* gene (Fig. 7). Mutant cells (*Ala6-nsp1::HIS3, nup82::HIS3*) carrying plasmid pCH1122-URA3-NSP1 and pUN100-LEU2-ProtA-NUP82Δ624-713 could grow at 23°C, but stopped growth on FOA-containing plates (Fig. 7). Synthetic lethality, however, was complemented by the presence of pRS314-ProtA-NUP82 (Fig. 7). This shows that the combination of mutant alleles of *NSP1* and *NUP82* functional at 23°C can cause synthetic lethality. Thus, Nsp1p and Nup82p not only physically but also functionally interact via their carboxy-terminal domains.

Nucleocytoplasmic Transport and Nuclear Envelope Structure in Yeast Cells Depleted of Nup82p

The fact that Nup82p physically interacts with Nsp1p and can be found at the NPC prompted us to test whether it plays a role in nucleocytoplasmic transport and/or nuclear envelope organization. It was shown earlier that repression of *NSP1* gene expression using a GAL::nsp1 strain caused cytoplasmic accumulation of the Matα2-lacZ nuclear reporter protein (a fusion protein consisting of the yeast transcriptional repressor Matα, which contains a nuclear localization sequence, and *Escherichia coli* β-galactosidase) and a decrease in NPC density (Mutvei et al., 1992). When the GAL::nup82 strain was tested in a similar way, cells did not reveal a clear defect in nuclear uptake of Matα2-lacZ after 9 h of glucose repression (Fig. 8 *A*). However, when analyzed for nuclear poly(A)$^+$ RNA export by in situ hybridization using a FITC-labeled oligonucleotide poly(dT)$_{50}$ probe (Kadowaki et al., 1992; Amberg et al., 1992), GAL10::ProtA-nup82 cells showed a time-dependent increase in poly(A)$^+$ RNA accumulation inside the nucleus upon shift to the restrictive growth medium. After 6 h in glucose medium, about 30% of the cells showed intranuclear poly(A)$^+$ RNA accumulation (data not shown) and after 9 h almost all of the cells revealed this defective phenotype (Fig. 8 *B*, *GLU*). This matches

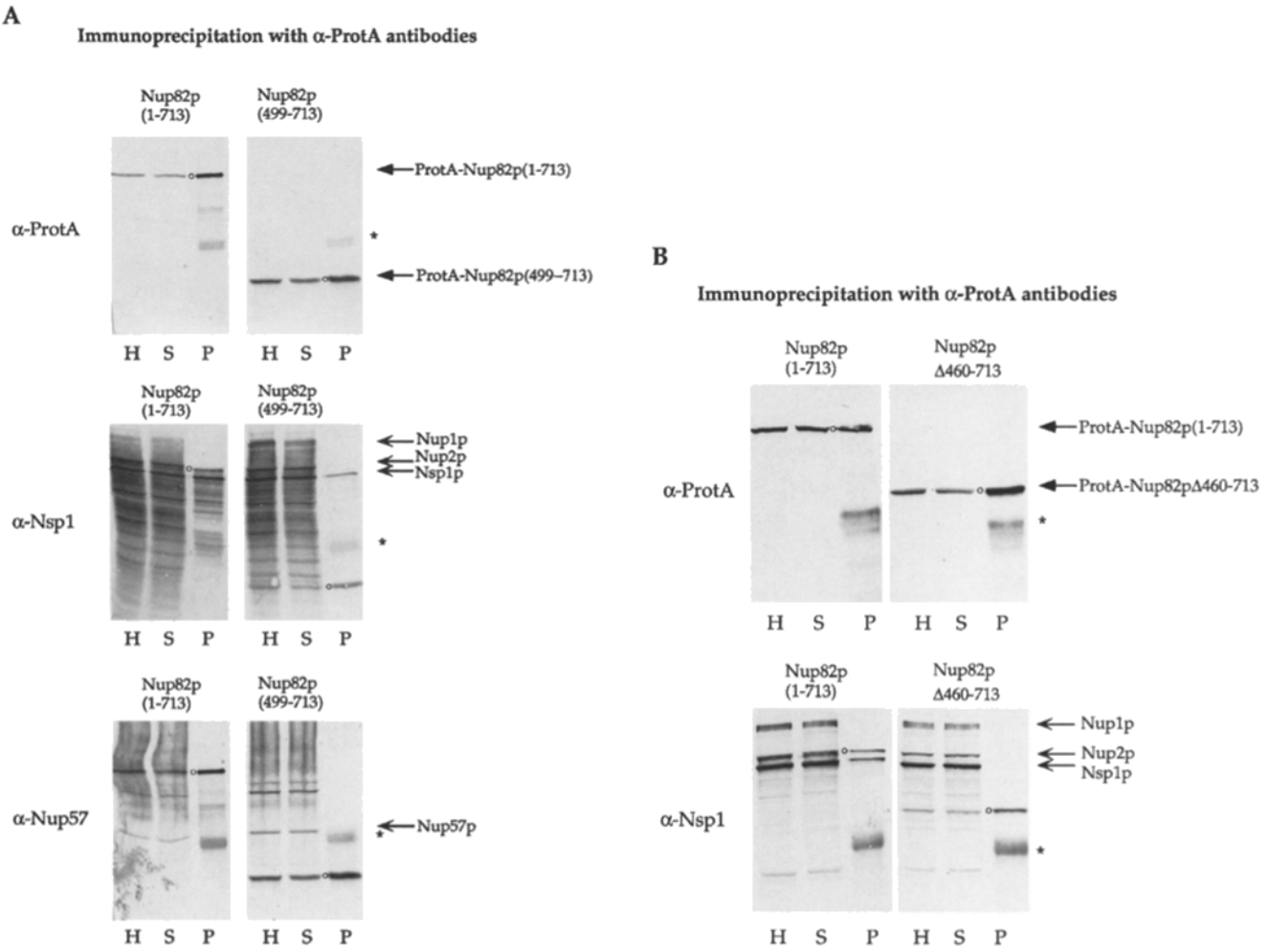

Figure 6. Native immunoprecipitation of mutant forms of Nup82p. (*A* and *B*) Immunoprecipitation from cell lysates expressing tagged wild-type ProtA-Nup82p(1-713) or ProtA-Nup82p(499-713) (*A*), and ProtA-Nup82pΔ460-713 or ProtA-Nup82p(1-713) (*B*) under non-denaturing conditions was done as described under Materials and Methods. A onefold equivalent of the homogenate (*H*) and immune supernatant (*S*) and a fivefold equivalent of the immune pellet (*P*) were analyzed by SDS-PAGE and Western blotting. Blots were probed with IgG coupled to HRP (*α-ProtA*), anti-Nsp1 (*α-Nsp1*) and anti-Nup57 (*α-Nup57*; made against the Nup57p carboxy-terminal domain) antibodies. The position of the bands is indicated by arrows. In addition, the circles show the position of the various ProtA-Nup82p fusion proteins. The star marks IgG heavy chain. Note that anti-Nsp1 antibodies cross-react with Nup1p and Nup2p and both anti-Nsp1 and anti-Nup57 antibodies also recognize the ProtA-fusion proteins. The band corresponding to Nup2p (*α-Nsp1*), although very closely migrating, is distinct from the band corresponding to ProtA-Nup82p(1-713) as it can be better appreciated in lane H and S of (*B*) and Nup2p does not co-precipitate with any of the fusion proteins.

well with the depletion of ProtA-Nup82p which is still detectable after 6 h, but not after 9 h of depletion (see also Fig. 3 *C*). In contrast, GAL10::nsp1 cells grown for 12 h in glucose medium did not exhibit such a deficiency in poly(A)$^+$ RNA export (Fig. 8 *B*). These data indicate that Nup82p expression, in contrast to Nsp1p expression, is required for efficient poly(A)$^+$ RNA export from the nucleus.

To find out whether depletion of Nup82p from yeast cells causes structural abnormalities of the nuclear envelope or a decrease in the NPC number, electron microscopic analysis of fixed and thin-sectioned GAL::nup82 cells grown for 9 h in glucose-containing medium was performed (Fig. 9). Although no effort was made to precisely quantify NPCs in the Nup82-depleted cells, no striking decrease in the nuclear pore number was noticed as compared to control cells (Fig. 9). The NPCs were also not altered in terms of their morphological appearance. Furthermore, no gross ultrastructural changes of the nuclear envelope such as NPC clustering or nuclear envelope herniations and invaginations as described for other nucleoporin mutants (Wente and Blobel, 1993; Doye et al., 1994; Wente and Blobel, 1994) were found in most of the observed cells.

Discussion

Upon defining the physico-functional interactions of proteins at the nuclear pore complex, one might also have clues as to how nucleocytoplasmic transport occurs on a molecular level. It is therefore important to isolate nuclear

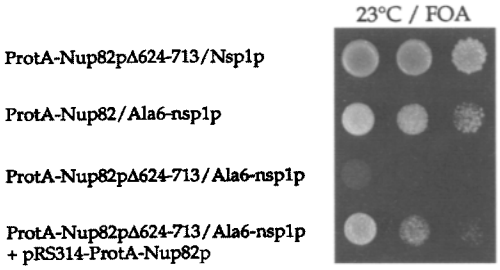

Figure 7. Synthetic lethality between Ala6-nsp1p and carboxy-terminally truncated Nup82pΔ624-713. Single and double nsp1 and nup82 mutant strains were constructed as described under Materials and Methods; these include the NUP82 shuffle strain complemented by pUN100-ProtA-NUP82Δ624-713 but lacking pRS316-URA3-ProtA-NUP82, the Ala6-nsp1 strain, the Ala6-nsp1/ProtA-NUP82Δ624-713 double mutant strain containing the pCH1122-URA3-NSP1 plasmid and the latter strain transformed with the pRS314-TRP1-ProtA-NUP82 plasmid. For each strain the same amount of cells derived from 1/10 and 1/100 dilutions from YPD (rich glucose media) liquid cultures were spotted onto 5-FOA plates and incubated for three days at 23°C. By this "dot spotting," the relative growth rates and cell viability of the used strains can be directly seen on the plate.

pore proteins under non-denaturing conditions and identify components with which they interact. These interacting proteins may be either intrinsic constituents of the NPC or transport factors which transiently associate with nuclear pore proteins, e.g., during translocation of substrates through the NPC.

We have purified the nuclear pore protein Nsp1p from yeast in order to identify its interacting partners. Nsp1p plays a role in uptake of nuclear proteins (Mutvei et al., 1992; Nehrbass et al., 1993), but is also linked to components which are involved in poly(A)$^+$ RNA export (Doye et al., 1994). Recently, we found that Nsp1p forms a stable complex with three other nuclear pore proteins Nup49p, Nup57p, and Nic96p (Grandi et al., 1993). Mutations in Nsp1p, Nup49p, and Nic96p which weaken physical interactions in this complex also impair nuclear import (Nehrbass et al., 1993; Doye et al., 1994; Grandi et al., 1995). Here, we demonstrate that another fraction of Nsp1p which is not associated with the members of the previously characterized complex is physically attached to a novel NPC protein, Nup82p. This shows that Nsp1p is present in distinct subcomplexes of the NPC.

The essential Nsp1p carboxy-terminal coiled–coil domain is sufficient for the interaction with both the nucleoporin complex consisting of Nup49p, Nup57p, and Nic96p as well as with the heptad repeats containing carboxy-terminal domain of Nup82p. How can the same Nsp1p carboxy-terminal domain bind to at least two different proteins? Interestingly, the continuity of the heptad repeat pattern in the Nsp1p carboxy-terminal domain is interrupted roughly in the middle of this domain (Hurt, 1990). We have reported earlier that mutations in the first half of the Nsp1p carboxy-terminal domain impair interaction

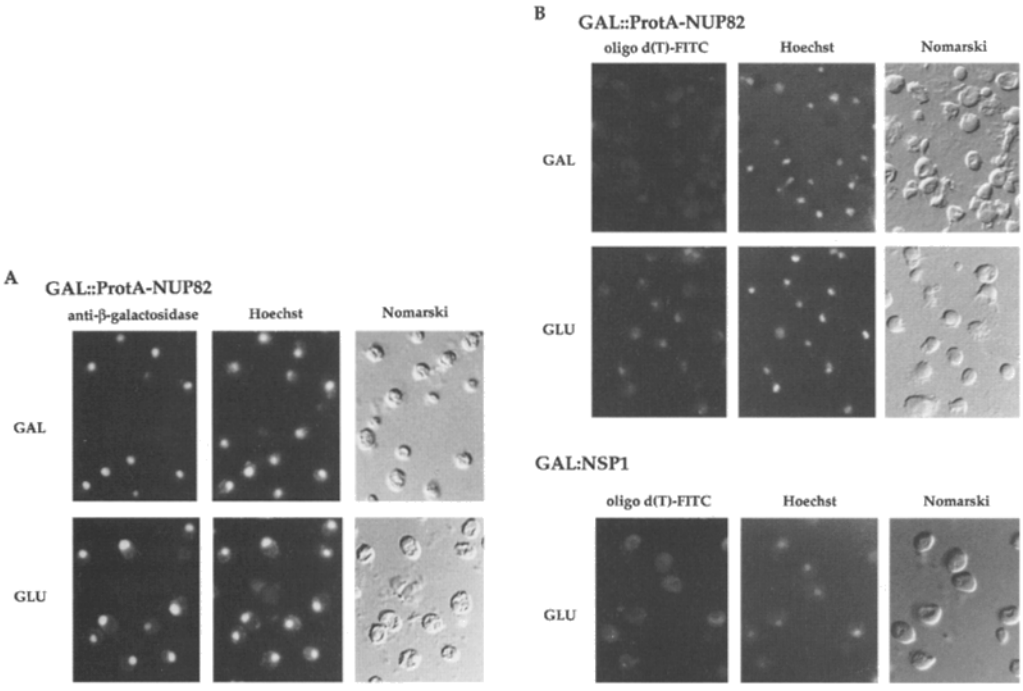

Figure 8. Nucleocytoplasmic transport in Nup82p depleted cells. (*A*) Nuclear import of Matα2-lacZ. GAL10::ProtA-NUP82 cells were transformed with pRS316-Matα2-lacZ on SGal −ura plates and transformants were grown either in SGalC −ura medium (galactose) or shifted for 9 h to SDC −ura medium (glucose). The location of the Matα2-lacZ reporter was analysed by immunofluorescence on spheroplasted cells using anti-β-galactosidase antibodies (1::100 dilution) followed by FITC-labeled goat anti-mouse IgG (1:100 dilution). Also nuclear DNA and cell morphology were viewed. (*B*) Poly(A)$^+$ RNA export. Subcellular localization of poly(A)$^+$ RNA was analysed in GAL10::ProtA-NUP82 cells and GAL10::NSP1 cells by in situ hybridization with a FITC-labeled oligonucleotide poly(dT)$_{50}$ probe. GAL10::ProtA-NUP82 and GAL10::NSP1 strains were grown in galactose or for 9 or 12 h in glucose-containing medium, respectively. Nuclear DNA was stained by Hoechst 33258 and cells were viewed by Nomarski optics.

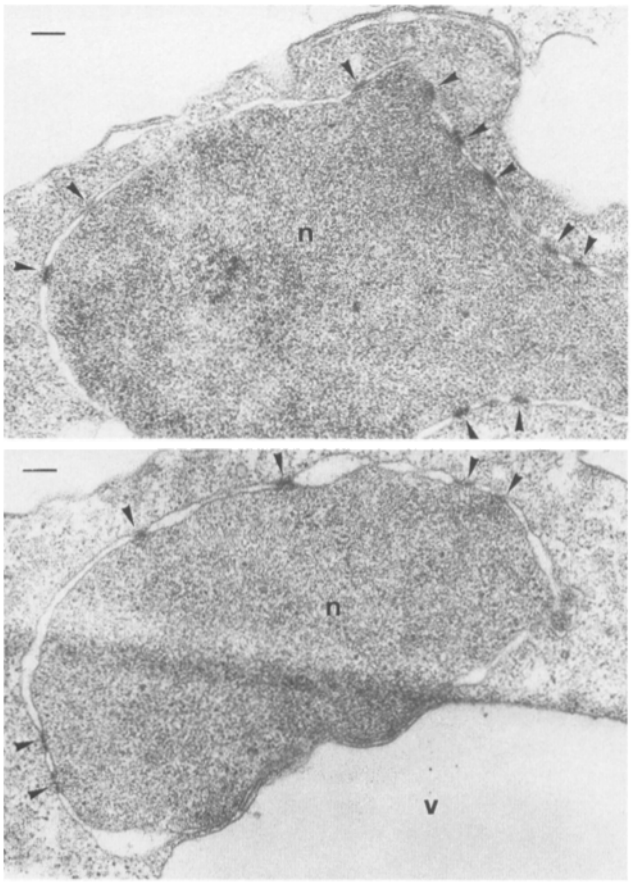

Figure 9. Thin-section electron microscopy of Nup82p depleted cells. GAL::nup82 cells grown for 9 h in glucose containing YPD medium at 30°C to deplete Nup82p were processed for thin section electron microscopy as described under Materials and Methods. Two EM photographs of the same mutant are shown. Arrowheads indicate nuclear pore complexes. *n*, nucleus; *v*, vacuole. Bars, 0.2 μm.

with Nic96p, but Nup82p still remained associated with Nsp1p (Grandi et al., 1993). It is therefore possible that the heptad repeats in the second half of the Nsp1p carboxy-terminal domain participate in the interaction with Nup82p. We are in the process of testing this by in vitro assembly of the purified proteins and their derived mutant forms.

Interestingly, also vertebrate p62, which is the presumptive homologue of Nsp1p (Carmo-Fonseca et al., 1991), seems to be organized in separate complexes: in a 600-kD complex together with a p58 and p54 component (which could be the Nup57p and Nup49p homologues, respectively) and a 1 MDa complex together with a p200 component which binds to wheat germ agglutinin (Macaulay et al., 1995).

The reason for multiple interactions of a single nucleoporin is not clear, but in this way nucleoporins may be linked to several NPC structures or different factors involved in nucleocytoplasmic transport (e.g., factors involved in nuclear protein or snRNP import or export of mRNA, snRNA, tRNA, or rRNA). Transcriptional repression of *NUP82* results in accumulation of poly(A)$^+$ RNA inside the nucleus before any defect in nuclear protein import, whereas lack of Nic96p from the cell impairs the import of proteins into the nucleus, but mRNA export is normal (Grandi et al., 1995). Thus, Nsp1p is associated with two nuclear pore proteins which do not physically interact with each other and are somehow involved in different transport pathways at the NPC. Interestingly, in vivo experiments showed that Nsp1p interacts also genetically with a large number of nuclear pore proteins involved not only in nuclear protein import, but also in mRNA export (Doye et al., 1994) and tRNA biogenesis (Hurt, E. C., unpublished data).

Yeast Srp1p, localized at the NPC (Yano et al., 1992), forms two distinct complexes with Nup1p and Nup2p (Belanger et al., 1994). Since vertebrate importin, which is one of the key components in NLS-mediated nuclear protein uptake, is homologous to yeast Srp1p (Görlich et al., 1994), in this case Nup1p and Nup2p might form two separate docking sites for the same NLS receptor-dependent protein import pathway at the NPC.

It will be interesting to find out whether a single nucleoporin can be located at different sites of the nuclear pore complex, i.e., at the cytoplasmic side which has access to the nuclear protein uptake system, and the inner side linked to factors involved in RNA export. Although we do not know the exact location of Nsp1p at the NPC, it may be part of the central pore structures (e.g., the plug or transporter) by analogy to nucleoporin p62 (see also above) which was sublocalized to both sides of the central pore channel (for review see Fabre and Hurt, 1994; Peters, R., personal communication). Experiments are under way to find out whether the two different Nsp1p-containing complexes are localized at different sites of the yeast NPC and whether the purified or in vitro reconstituted complexes exhibit distinct structures at the electron microscopic level.

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