***NUP2*, a Novel Yeast Nucleoporin, has Functional Overlap with Other Proteins of the Nuclear Pore Complex**

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We have isolated a new gene, *NUP2*, that encodes a constituent of the yeast nuclear pore complex (NPC). The *NUP2* protein sequence shares a central repetitive domain with *NSP1* and *NUP1*, the two previously characterized yeast nucleoporins. Like *NUP1* and *NSP1*, *NUP2* localizes to discrete spots in the nuclear envelope, as determined by indirect immunofluorescence. Although the sequence similarity among these three nucleoporins suggests that they have a similar role in the nuclear pore complex, *NUP2*, in contrast to *NSP1* and *NUP1*, is not required for growth. Some combinations of mutant alleles of *NUP1*, *NSP1*, and *NUP2* display “synthetic lethal” relationships that provide evidence for functional interaction between these NPC components. This genetic evidence of overlapping function suggests that the nucleoporins act in concert, perhaps participating in the same step of the recognition or transit of macromolecules through the NPC.

INTRODUCTION

Because the nuclear envelope acts as a barrier to separate nuclear metabolism (e.g., transcription) from cytoplasmic metabolism (e.g., translation), transit of molecules between these compartments must occur via a specialized organelle, the nuclear pore complex (NPC).¹ The bidirectional traffic of macromolecules through the NPC has been shown to be a specific concerted process (for review see Garcia-Bustos *et al.*, 1991a; Silver, 1991). Conservation of the ultrastructure of the NPC among many species (Newport and Forbes, 1987) suggests that this organelle operates via the same mechanism in all eukaryotes. The prevailing model for nuclear import of proteins and small nuclear ribonucleoproteins involves recognition by cytoplasmic receptors, receptor/substrate docking at the NPC, and either transport of the complex or release of the substrate to the pore apparatus for transport. Substrate recognition occurs via multiple receptors (Fisher *et al.*, 1991; Garcia-Bustos *et al.*, 1991b;

Michaud and Goldfarb, 1991), some candidates for which have been identified (Adam *et al.*, 1989; Lee and Mélése, 1989; Silver *et al.*, 1989; Adam and Gerace, 1991). The translocation step is an ATP-dependent process (Newmeyer and Forbes, 1988; Richardson *et al.*, 1988), but the ATP binding and hydrolyzing components have not yet been identified. The reciprocal transport of mRNA and ribosomes from the nucleus to the cytoplasm is less well understood. It appears that transport to the cytoplasm is intertwined with RNA processing steps within the nucleus and that the transport apparatus itself might not be responsible for specificity (reviewed in Davis, 1992).

The NPC is a proteinaceous assembly of over 10⁸ Da, and it has been estimated to be composed of over 100 polypeptides (Reichelt *et al.*, 1990). The majority of the NPC constituent proteins are yet to be identified. Most NPC proteins that have been characterized thus far belong to a family of immunologically related proteins (nucleoporins) first identified by monoclonal antibodies raised against mammalian NPC antigens (Davis and Blobel, 1986, 1987; Park *et al.*, 1987; Snow *et al.*, 1987). Some nucleoporins have been shown by immunoelectron microscopy to be limited to the central “trans-

¹ Abbreviations used: 5-FOA, 5-fluoroorotic acid; HA, hemagglutinin; kb, kilobase; MAb, monoclonal antibody; NPC, nuclear pore complex; ORF, open reading frame; SC, synthetic complete; WGA, wheat germ agglutinin.

porter" region of the NPC, the site where the transport mechanism is suspected to reside (Akey and Goldfarb, 1989). In vertebrates, three nucleoporin proteins have been shown to exist as a complex of ~500 kDa (Finlay *et al.*, 1991). Genes for three nucleoporins have been isolated: p62 from mammals and *NUP1* and *NSP1* from budding yeast (Davis and Fink, 1990; Nehrbass *et al.*, 1990; Starr *et al.*, 1990, respectively). The immunological cross-reactivity of these proteins is probably due to a repetitive sequence motif. This motif, which is not identical in each protein, consists of a series of a degenerate nonapeptides each separated by a less-conserved highly charged spacer sequence. Many, if not all, of these proteins are also recognized by the lectin wheat germ agglutinin (WGA) by the virtue of the fact that they contain multiple O-linked N-acetyl glucosamine residues.

Several lines of evidence suggest that nucleoporins are directly involved with nuclear transport. WGA inhibits the translocation step of protein import both in vitro and in vivo (Finlay *et al.*, 1987; Dabauvalle *et al.*, 1988). Furthermore, depletion of the WGA binding proteins from *Xenopus* cytosolic extracts before nuclear envelope reconstitution prevents even the initial binding of a transport substrate to the NPC but does not affect passive diffusion (Finlay and Forbes, 1990). In addition, WGA binding proteins appear to bind a cytoplasmic factor that is required for the transport of proteins through the nuclear pore in an in vitro assay, suggesting that nucleoporins might serve as "docks" for receptor-substrate complexes (Sterne-Marr *et al.*, 1992). Furthermore, the yeast *NSP1* protein has been implicated in the transport of proteins across the nuclear envelope, because a conditional allele mislocalizes nuclear proteins at the nonpermissive temperature (Nehrbass *et al.*, 1990).

The sequences of *NUP1* and *NSP1* suggest a common tripartite pattern for yeast nucleoporins: each protein consists of unique terminal sequences flanking the central repeated motif recognized by the monoclonal antibodies. Not all of these sequence segments are required for viability. For example, a strain containing an *NSP1* disruption is viable if supplied with a construct containing the 217 amino acid carboxy-terminal region of *NSP1*, lacking the central repeated motif. This carboxy-terminal domain is sufficient to target a heterologous protein to the nuclear envelope (Hurt, 1990). The dispensability of the repeated domain of *NSP1* indicates that the repeated motif of nucleoporins might have a common or redundant function, whereas the requirement for the unique carboxy-terminal region suggests that each nucleoporin may have a unique role in NPC function.

To address the role of nucleoporins in nuclear transport and to pursue the significance of the unusual domain structure of the nucleoporin gene family, we have sought additional genes that contain the repetitive do-

main common to *NUP1* and *NSP1*. This search led to the isolation of a third related gene from yeast, *NUP2*. In contrast to *NUP1* and *NSP1*, *NUP2* is not essential for growth. However, in conjunction with mutations in either the *NUP1* or *NSP1* genes, *NUP2* is required. We have utilized the synthetic phenotypes of combinations of mutations in these genes to assess the significance of the conserved and divergent domains of this gene family.

MATERIALS AND METHODS

Reagents

Enzymes for molecular biology were purchased from New England Biolabs (Beverly, MA), Pharmacia (Piscataway, NJ), and Promega (Madison, WI). Yeast spheroplasts were prepared using oxalyticase (Enzogenetics, Corvallis, OR) for immunofluorescence, and zymolyase 100T (Kirin Brewing, Takasaki, Japan) was used to prepare asci for tetrad dissection. Secondary antibodies were obtained from Jackson ImmunoResearch (Westgrove, PA). Radiochemicals were purchased from Amersham (Arlington Heights, IL), and all other chemicals were obtained from Sigma (St. Louis, MO).

DNA Techniques

DNA subcloning was done by standard techniques outlined in Sambrook *et al.* (1989). A complete list of plasmids used in this study is shown in Table 1.

Cloning Potential Nucleoporin Genes

In previous studies, a yeast genomic expression library was screened with MAB350 (Davis and Fink, 1990). Thirteen unique positive clones (including *NUP1*) were identified. Protein was prepared from lysogenic *Escherichia coli* strains harboring the positive lambda clones and subsequently immunoblotted with a series of monoclonal antibodies to mammalian NPC antigens to identify the clones that express authentic nucleoporin epitopes. As a second test, Southern blots of plasmid DNA subcloned from each positive lambda clone were probed at low stringency with a degenerate oligonucleotide (deNSP1) complementary to all possible nucleotide sequences that can encode the peptide sequence (KPAFSFGAK), precisely conserved in 12 of 22 of the repeated units in *NSP1* (sequence of deNSP1: 5'(A/T)TT IGC ICC (A/G)AA IGA (A/G)AA IGC IGG (C/T)TT^{3'} where positions in parentheses are equal mixtures of indicated nucleotides and I indicates inosine). Hybridization conditions were as follows: 5.5× SSC (825 mM NaCl, 83 mM sodium citrate), 5× Denhardt's solution, 1% sodium dodecyl sulfate, 0.2 mg/ml calf thymus DNA, 1 mM EDTA. Blots were incubated with end-labeled oligonucleotide probe for 18 h at temperatures of either 40, 50, or 65°C, then briefly washed at room temperature in 6× SSC, 1% sodium dodecyl sulfate, dried, and exposed to x-ray film.

Full-length clones of four genes that were positive in either the immunoblotting or the hybridization tests were isolated from a genomic plasmid library in the yeast shuttle vector YEp24 (Carlson and Botstein, 1982) by colony hybridization (Sambrook *et al.*, 1989) with RNA probes synthesized in vitro from the plasmid subclones. These four plasmids were tested by the overexpression assay detailed in RESULTS. One clone (pJON35) was identified as *NUP2*. The three other lambda clones tested by the overexpression assay failed to show overexpression of a polypeptide recognized by the monoclonal antibodies. Because this approach depends on isolation of the full-length gene and its subsequent overexpression, either the YEp24 isolates identified by these lambda clones did not contain the full-length gene or expression was modulated by unknown means in the constructs assayed.

Table 1. Plasmids used in this study

Yeast plasmid	Relevant markers	Comments	Source
pB2201	2 μ LEU2	Derived from pRS305 by insertion of 2 μ sequences at AatII site	D. Miller
pB2204	CEN LEU2	GAL 1-10 promoter inserted into pRS315 ^a	D. Miller
pJON35	2 μ URA3 NUP2	13-kb fragment containing NUP2 from YEp24 library	This study
pJON37	2 μ LEU2 NUP2	6.2-kb BamHI fragment containing NUP2 in pB2201	This study
pJON73	CEN LEU2 NUP2	6.2-kb BamHI fragment containing NUP2 in pRS315	This study
pJON80	CEN LEU2 NUP2::HA	HA epitope inserted at aa. 428 by oligo mutagenesis of pJON73	This study
pJON86	CEN LEU2 GAL::NUP2	NUP2 coding sequence under GAL promoter in pB2204	This study
pB2487	CEN URA3 NUP1	NUP1 wild-type inserted into pRS316	This study
pB2289	CEN TRP1 NUP1	NUP1 wild-type in pRS314	This study
pB2291	CEN TRP1 nup1-8 (Δ 4-141)	Internal deletion of pB2289 created by exoIII digestion	This study
pB2300	CEN TRP1 nup1-21 (Δ 1042-1076)	Internal deletion of pB2289 created by exoIII digestion	This study
pJON139	CEN TRP1 nup1-8 NUP2	Wild-type NUP2 inserted into pB2289	This study
pJON146	CEN TRP1 nup1-8 NUP2::HA	NUP2::HA inserted into pB2289	This study
pJON164	CEN TRP1 nup1-8 nup2-9	nup2-9 (Δ 86-720) inserted into pB2289	This study
pJON149	CEN TRP1 nup1-8 nup2-10	nup2-10 (Δ 175-720) inserted into pB2289	This study
pJON165	CEN TRP1 nup1-8 nup2-11	nup2-11 (Δ 556-720) inserted into pB2289	This study
pB2486	2 μ LEU2 NSP1	Wild-type NSP1 in YEp13	Nehrbass <i>et al.</i> , 1990
pB2485	CEN LEU2 C-NSP1	NSP1 carboxy-terminus under ADH promoter	Nehrbass <i>et al.</i> , 1990
pB2483	2 μ LEU2 NSP1 (Δ 173-605)	Derived from pB2486	Nehrbass <i>et al.</i> , 1990
<i>E. coli</i> plasmids	Description		Source
pJB107	pUC118 with HindIII site destroyed by filling in and religation		J. Brill
pLD15.1	a 3-kb EcoRI fragment from λ gt11 clone 15.1 subcloned into pGEM7		This study
pJON12	NUP2::URA3: 2-kb Sac I fragment of pLD15.1 in pRS306		This study
pJON53,54	NUP2 6.2-kb BamHI fragment pBluescript ks(+) inserted in both orientations		This study
pJON107	NUP2 4kB Pst I to BamHI in pJB107 mutagenized as per text		This study
pJON113	nup2-4::URA3 derived from pJON107		This study
pJON115	nup2-5::HIS3 derived from pJON107		This study
pJON133	nup2-7::TRP1 derived from pJON107		This study
pJON166	nup2-12::TRP1 created by replacing internal Apa I fragment of NUP2 with TRP1		This study
pB2337	nup1-2::LEU2 created by replacing region from SnaBI to BspMI of NUP1 with LEU2		This study
pB2482	nsp1::URA3 disruption construct		Nehrbass <i>et al.</i> , 1990

^a pRS vectors are from Sikorski and Heiter (1989). All plasmids are amp^r.

DNA Sequencing

Nested series of deletions were made from plasmids pJON53 and pJON54 by a modification of the ExoIII protocol of Henikoff (1984), substituting ExoVII nuclease for S1 nuclease. Double-stranded dideoxy sequencing with Sequenase was performed as per manufacturer's (United States Biochemical, Cleveland, OH) instructions. The sequence was edited using the Lasergene package (DNASTAR, London, UK), analyzed using UWGCG programs (Devereux *et al.*, 1984), and sequence searches were performed using the NCBI BLAST (Bethesda, MD) network service. We sequenced through two neighboring genes upstream of the NUP2 coding sequence. One hundred eighty nucleotides 5' from the first potential start site for NUP2 is the tRNA^{GLU}3 CYH82 with its associated TY δ element distal to NUP2 (Hauber *et al.*, 1988). Further upstream is an open reading frame (ORF) with very high homology to the S31 ribosomal protein of *Saccharomyces carlsbergensis* (Nieuwint *et al.*, 1985).

Site-Directed Mutagenesis

Mutants were made by the single-stranded mutagenesis technique of Kunkel *et al.* (1987). dut⁻ ung⁻ strain RZ1032 was used to isolate

single-stranded plasmid DNA, synthesis was performed as described (Kunkel *et al.*, 1987), and the reaction mixture was transformed by the CaCl₂ technique into *E. coli* strain DH5 α .

The complete disruption constructs of NUP2 were made by mutagenizing plasmid pJON73 with two oligos to introduce a series of restriction sites just outside of the initiation and termination codons of NUP2. The 5' oligo was of sequence CTCAAAAAAATCATTAAC-GAGGATATCTAGACTAGTATGGCCAAAAGAGTTGCCG and the 3' oligo was of sequence GCTAAAAAAGAAATGAAATAAACTA-GTCTAGATATCGAGTAAATACAGTTAACAATTTTAAATAG. A BamHI/Pst I fragment containing the mutagenized NUP2 was subcloned into pJB107, and then the NUP2 coding region of the resulting plasmid, pJON107, was replaced by selectable markers by standard cloning techniques to yield pJON113, pJON115, and pJON133. The HA antigen was introduced into NUP2 by mutagenesis of pJON73 with a oligo of sequence GGTACCGAGAAAGAGAAGTACCCAT-ACGACGTCCTCCAGACTACGCTGAAAGTAAAAAGCACTCA. The C-terminal truncations of NUP2, nup2-9, nup2-10, and nup2-11, were created by introducing stop codons in plasmid pJON139 at the positions indicated in the text with the following oligos: GCTCAGGTTGATAATAGCCCTTACCCATACGACGTCCAG-ACTACGCTATGAAATAATAGTGACCGCGGACCCACAGAA-

AGCAATTCCAGA, GATGAGGTAAAGGTGGAGGGGTACCC-ATACGACGTCCAGACTACGCTTAATAGTGACCCAAAGTTC-ACAATAGATGCT, and GGGTTCAAATTTCTTTGCCATACC-CATACGACGTCCAGACTACGCTATGAAATAATAGTGAC-CGCGGTTTGAACAAAAAGGTAGTCAA, respectively.

Yeast Growth Conditions and Strain Construction

Yeast cell culture and crosses were performed essentially as outlined in Sherman *et al.* (1986). Yeast plasmids and linear DNA fragments for integration were transformed into yeast by lithium acetate transformation (Ito *et al.*, 1983). Selection against *Ura*⁺ strains was done by culture on solid synthetic media containing 1 mg/ml 5-fluoroorotic acid (5-FOA) as described in Boeke *et al.* (1984). A complete list of strains used in this study is shown in Table 2.

Construction of the strain (JLY25) bearing the *NUP2* gene marked with *URA3* for mapping was performed as follows: strain L2612 was transformed with a plasmid (pJON12) that contains approximately one half of *NUP2*, from the internal *Sac* II fragment to beyond the 3' end of the coding sequence in the vector pRS305 (Sikorski and Heiter, 1989). This plasmid contains the yeast *URA3* gene but lacks a yeast origin of replication. pJON12 was linearized in the *NUP2* coding sequence with *Hpa* I and selected integrants on synthetic media lacking uracil. The positive transformants bear *URA3* flanked by a partial duplication of *NUP2* on one side and wild-type *NUP2* on the other.

RESULTS

Isolation of the Gene for a Novel Yeast Nucleoporin

A previous screen for yeast nucleoporins of a yeast *λgt11* phage expression library with the monoclonal antibody 350 yielded 12 independent positive clones in addition to *NUP1* (Davis and Fink, 1990). Four were chosen for

further analysis on the basis of the reactivity of their β -galactosidase fusion proteins expressed in *E. coli* with our panel of monoclonal antibodies to nuclear pore proteins (Davis and Blobel, 1986; Davis and Fink, 1990) and also by their homology to the cloned nucleoporins as assessed by low stringency Southern hybridization. One phage clone, λ 15, was very strongly recognized by a degenerate oligonucleotide probe (deNSP1; MATERIALS AND METHODS), the sequence of which was designed to identify the repeated element of the *NSP1* gene. This probe also hybridized to a *NUP1* plasmid but none of the other phage encoding putative nucleoporins.

We further examined these four clones to identify those that expressed proteins in yeast corresponding to novel nuclear proteins recognized by our panel of monoclonal antibodies (MAbs). Our test was as follows. High copy plasmids containing sequences corresponding to the promising lambda clones were isolated from a library of yeast genomic DNA in the high copy yeast shuttle vector YEp24 (Carlson and Botstein, 1982) by hybridization. These YEp plasmids were then introduced in the haploid yeast strain L2612, and total protein was immunoblotted with MAbs 306, 350, and 414 (Figure 1, A–C, respectively). The 2 μ origin of replication of YEp24 specifies very high copy number; therefore, a cloned gene under control of its own promoter should lead to enhanced expression of its protein relative to a vector control. Three major nuclear proteins react with

Table 2. Yeast strains

Strain name	Genotype	Source
L2612	<i>MATα ura3-52 leu2-3, 112 trp 1-289</i>	Fink collection
L3212	<i>MATα cdc25-1 ura3-52</i>	Fink collection
L3288	<i>MATα rna1-1 met trp1[^]1 ade2 ura3-52</i>	Fink collection
WLY401	<i>MATα nsr1-1::HIS3 lys2-801 ade2-101 trp1[^]1 his3[^]200 ura3-52</i>	Lee <i>et al.</i> , 1991
LDY53	<i>MATα ura3-52 leu2-3,112 trp1-289</i>	This study
JLY25	<i>MATα NUP2::URA3 ura3-52 leu2-3,112 trp1-289</i>	This study
JLY48	<i>MATα nup2-4::URA3 ura3-52 leu2-3,112 trp1-289</i>	This study
JLY242	<i>MATα nsp1::URA3 ura3-52 leu2-3,112 trp1-289 {pB2486}</i>	This study
L4745	<i>MATα nup1-2::LEU2 his3[^]200 trp1[^]1 ura3-52 leu2-3,112 {pB2487}</i>	This study
JLY119	<i>MATα nup1-2::LEU2 his3[^]200 trp1[^]1 ura3-52 leu2-3,112 nup2-5::HIS3 {pB2487}</i>	This study
JLY54	<i>MATα nup2-12::TRP1 ura3-52 leu2-3,112 trp1-289</i>	This study
JLX19	<i>MATα/MATα ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1-289/trp1-289</i>	This study
JLX48	<i>MATα/MATα NSP1/nsp1-URA3 ura3-52/ura3-52 leu2-3, 112/leu2-3,112 trp1-289/trp1-289</i>	This study
JLX49	<i>MATα/MATα NUP1/nup1-2::LEU2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1-289/trp1-289</i>	This study
JLX53	<i>MATα/MATα nsp1::URA3/NSP1 nup2-7::TRP1/NUP2 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1-289/trp1-289</i>	This study
JLX78	<i>MATα/MATα nup2-4::URA3/nup2-4::URA3 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1-289/trp1-289</i>	This study

All strains are of S288C background. Strains LDY53, JLY25, 48, 54, 242, and JLX19–JLX78 are isogenic to L2612. The L2612 isogenic series is also *can1* and *cyh1*. Strain JLY119 is isogenic to L4745. Brackets indicate resident yeast plasmids.

Figure 1. Overexpression of a novel nucleoporin gene. Western blots of total protein from the indicated strains were probed with anti-nucleoporin monoclonal antibodies as indicated. Lane 1, wild-type strain L2612, transformed with the YEp24 vector; lane 2, L2612 bearing the pJON35 plasmid; lane 3, L2612 bearing pB2486 (YEp13 *NSP1*) (Nehrbass *et al.*, 1990); lane 4, JLY241, a *nsp1::URA3* strain bearing pB2483 (YEp13 *NSP1Δ* 173-605). The ~50-kDa polypeptide recognized by the antibodies has been shown to be cytoplasmic (Davis and Fink, 1990). The dots at left correspond to the following molecular weight standards: myosin, 220 kDa; B-galactosidase, 116 kDa; phosphorylase b, 97 kDa; bovine serum albumin, 66 kDa. Yeast whole cell extracts were prepared by glass bead lysis directly into trichloroacetic acid (Ohashi *et al.*, 1982). Western transfer and antibody probing were done as described by Davis and Fink (1990) using 5% nonfat milk instead of 2% bovine serum albumin.

our MAbs (Davis and Fink, 1990). NUP1 migrates at 130 kDa and is detectable in whole cell extracts only with the 306 antibody. All three MAbs recognize a major doublet at 100 kDa that is comprised of NSP1 and an uncharacterized protein. Strains bearing plasmid (pJON35) clearly overexpress this 95-kDa protein, which is distinct from NSP1 (Figure 1, lanes 2 and 3). The pJON35 plasmid was isolated using the λ 15 clone as a probe. The presence of this protein in a strain bearing a truncated version of *NSP1* (Δ 173-605) (Nehrbass *et al.*, 1990) provides further evidence that this protein is not NSP1 nor its degradation product (Figure 1, lane 4). The truncated *NSP1* gene lacks the central repeated domain and is unreactive with our MAbs. Furthermore, we tested our overexpressing extracts with the 414 antibody (Figure 1C). Thereby we have shown that *NSP1* and the newly identified gene must encode the two yeast nucleoporin proteins originally characterized on the basis of their reactivity with this antibody (Aris and Blobel, 1989). *NSP1* encodes the protein Aris and Blobel refer to as p110, and the new gene encodes the p95 protein. It is also probable that this new gene encodes the protein previously referred to as NSP2 (Nehrbass *et al.*, 1990).

The 95-kDa Protein is Encoded by NUP2

Various fragments of the 13 kilobase (kb) insert of pJON35 were subcloned and assayed for overexpression of p95 by Western blot. Plasmid pJON37 contains a 6.1 kb *Bam*HI insert that maintains the overexpression phenotype. This *Bam*HI fragment was mapped and sequenced (Figures 2 and 3). The longest ORF in the sequence can encode a 77 954-Da 720 amino acid protein, assuming the first ATG is the initiator codon. This ORF shows similarity to *NUP1*, *NSP1*, and rat p62 (in order of similarity score when compared with the PIR, SWISS-PROT, and translated GenBank protein databases). On the basis of this homology and other evidence presented in subsequent sections, this gene was named *NUP2*. The only other significant matches appear to be a result of the serine-rich nature of the predicted *NUP2* sequence (13.5% serine as well as 12.3% lysine). Interestingly, the best match among serine rich proteins was with the *NSR1* gene, which encodes another yeast protein implicated in nuclear protein trafficking (Lee *et al.*, 1991). Although the predicted *NUP2* polypeptide is highly charged throughout its length, the predicted pI is 7.1. There are no putative transmembrane domains nor are there any regions particularly acidic or basic. No informative motifs were found when the sequence was compared against a library of ~400 well-characterized protein motif consensus sequences (Fuchs, 1991).

The position of *NUP2* on the physical and genetic maps of the yeast genome (Figure 3) was determined by hybridizing a labeled probe to a set of ordered λ phage clones (λ prime filters; Riles and Olson, unpublished data). The *NUP2* probe hybridized to a single clone, λ 6165, which is on chromosome XII and lies between the previously mapped genes *cdc3* and *ilv5*. To confirm this location, a haploid yeast strain (JLY25) containing the *NUP2* locus marked with the *URA3* gene was constructed. JLY25 was crossed with the *cdc25-1* strain L3212 (*cdc25* is just centromere proximal to *cdc3*) and 56 tetrads were dissected, yielding 44 parental ditypes, 8 tetratypes, and 0 nonparental ditypes, locating *NUP2::URA3* ~8 cM from *cdc25-1*. The orientation of the coding region of *NUP2* relative to the centromere of chromosome XII was determined by comparing our restriction map and sequence with the *Eco*RI/*Hind*III physical map of Riles and Olson (unpublished data).

The Repeated Domain Defines a Family of Nucleoporins

The homologies detected between the other nucleoporin protein sequences and the *NUP2* sequence are limited entirely to their repeated domains (Figure 4). We assigned the repeats of *NUP2* by plotting the regions of extreme hydrophobic moment in the β -sheet conformation using the algorithm of Eisenberg *et al.* (1984) and by analogy to the other nucleoporins. *NUP2* has 16 repeats (indicated by underline in Figure 2) in com-

Figure 2. Nucleotide and predicted protein sequence of the *NUP2* gene. Nucleoporin repeated motifs are underlined and the central phenylalanine-polar-phenylalanine stretch is in bold face. The EMBL accession number for the *NUP2* nucleotide sequence is X69964.

parison with 28 in *NUP1* and 22 for *NSP1*. *NUP2* lacks an uncharged domain, which is present at the carboxy terminus of *NUP1* and at the amino terminus of *NSP1*. Both *NUP2* and *NSP1* have several sequences at their amino termini that are similar to the repeated motif. Because the only sequence similarity shared between all four proteins is the repeats, it is very likely that this

sequence defines the common epitope between these proteins. The hallmark of the repeats is a pair of phenylalanines flanking a polar residue, most commonly serine (Figure 2 in boldface). This tripeptide sequence contributes most of the amphipathic nature to the repeats. Within *NUP1*, *NUP2*, and p62, the repeats follow a loose primary sequence homology, whereas the re-

Figure 3. Physical and genetic map of *NUP2* locus. Top, restriction map of *NUP2* region. The endpoints are equivalent to the ends of the insert in plasmid pJON39. Bottom, partial genetic map of chromosome XII indicating the position of *NUP2* relative to neighboring loci.

peating unit of *NSP1*, as well as its interrepeat spacers, is well conserved from one unit to the next. *NSP1* and p62 have a second region of homology, residues 350–520 of p62 and residues 610–820 of *NSP1*. It has been shown that the repeats of *NSP1* are dispensable for function (Nehrbass *et al.*, 1990), whereas it is unknown whether the repeated domain of *NUP1* is required.

NUP2 is a Component of the Nuclear Envelope

The intracellular localization of *NUP2* was determined by indirect immunofluorescence. As none of our MAbs is absolutely specific for *NUP2*, we constructed a *NUP2* derivative containing a DNA insert that encodes the nine amino acid influenza hemagglutinin (HA) antigen (Wilson *et al.*, 1984). This foreign epitope was placed at position 428 in the spacer region between the 9th and 10th repeat and results in a functional *NUP2* protein

(see subsequent section) that can be uniquely identified by the 12CA5 MAb directed against the HA epitope. This construct (pJON80) was expressed in a diploid strain lacking *NUP2* coding sequences (JLX78; described below), and the localization of the tagged protein was ascertained by indirect immunofluorescence (Figure 5A). Western blots of total protein from strains with epitope-tagged *NUP2* showed a specific 95-kDa band as well as a 60-kDa nonspecific band that could also be detected in strains lacking the tagged construct. The cross-reactivity of the 12CA5 antibody with this native 60-kDa protein probably contributes to the cytoplasmic background shown in controls containing only the vector (Figure 5C). The fluorescence staining of *NUP2* was reminiscent of that previously shown with an antibody specific for *NSP1* (Nehrbass *et al.*, 1990) and for epitope tagged *NUP1* (Davis and Fink, 1990) as well as anti-nucleoporin MAbs (Figure 5B). The antibody highlighted the nuclear envelope as a punctate brightly staining rim that surrounded the less intensely staining intranuclear region. No staining above background was evident in the cytoplasm. Also, in some fields, cells could be seen where staining was visible as the envelope extends into the bud neck early in mitosis and as a long process connecting daughter nuclei at a late stage in karyokinesis.

Strains Containing a Null Mutation of *NUP2* are Viable

Null mutations of *NUP2* were created by deleting the entire coding sequence of *NUP2* (MATERIALS AND METHODS) and replacing it with either *URA3*, *HIS3*, or *TRP1* (*nup2-4::URA3*, *nup2-5::HIS3*, and *nup2-7::TRP1*). The deletion/insertional alleles were integrated

Figure 4. Sequence homology between *NUP2* and other nucleoporins is confined to the repeated domain. Amino acid sequence matrix comparison derived from the UWGCG package COMPARE program: window 20, stringency 25. Vertical stripes indicate regions that have the canonical hydropathy profile of the repeated domain.

Figure 5. Immunofluorescence localization of the NUP2 protein. In each panel, a collage of cells from several fields are shown stained with the indicated MAb in the left column, whereas the corresponding DAPI stained nuclei of these cells are shown on the right. (A) *nup2Δ/nup2Δ* diploid strain JLX78 bearing the *NUP2::HA LEU2 CEN* plasmid pJON80 stained with MAb 12CA5 directed against hemagglutinin epitope. (B) Wild-type diploid strain JLX19 stained with anti-nucleoporin MAb 306. (C) JLX78 bearing a *LEU2 CEN* vector plasmid, as a negative control for antibody specificity, stained with 12CA5. Immunofluorescence on yeast spheroplasts was performed as described in Davis and Fink (1990) except that 1% polyethyleneimine in H₂O was substituted for poly-lysine and filtered 5% nonfat dry milk was used in place of 2% bovine serum albumin as the coating agent. Bar, 5 μ m.

by transformation to replace the resident *NUP2* allele on one of the two copies of chromosome XII in the diploid strain JLX19 (derived by mating the isogenic strains L2612 and LDY53). Transformants of each construct were sporulated, and tetrads were dissected. In $\sim 90\%$ of the tetrads, all four spores germinated, and

Nup2[−] spores were found at an equal frequency to *Nup2*⁺ spores. Hybridization experiments with one construct showed directly (Figure 6A) that the transformants have the *NUP2* coding sequence replaced by *URA3*. Strains bearing the marked *nup2* deletion alleles appear to lack any detectable NUP2 protein, because

Figure 6. Deletion of the *NUP2* coding sequence. (A) Southern blot of wild-type (+) and *nup2*Δ (–) strains. Genomic DNA from the parent strain (L2612) and from a strain transformed with the *nup2-4::URA3* construct (JLY48) was digested with *EcoRI*, electrophoresed, transferred to a nylon membrane, and then probed with a 0.4-kb *HindIII* fragment that contains the last 200 nucleotides of the *NUP2* coding sequence, as well as 200 nucleotides of 3' flanking sequence (see Figure 3). The alteration in mobility in the *nup2*Δ lane indicates a replacement of the 2.1-kb *NUP2* coding sequence with the 1.1-kb *URA3* gene. (B) Western blot of total protein from the same strains as shown in A probed with MAb 350.

levels of NUP1 and other uncharacterized cross-reactive proteins remain constant, whereas the NUP2/NSP1 doublet is diminished in intensity (Figure 6B).

The growth rate of the isogenic strains L2612 (*NUP2*) and JLY48 (*nup2-4::URA3*) on rich media at 30°C is identical. Because *nup2* deletion strains grow well under normal conditions, they were tested for other phenotypes such as temperature sensitivity, increased chromosome loss, inability to grow on nonfermentable carbon sources, and sensitivity to heat shock as well as carbon and nitrogen starvation. Strains carrying the *nup2* deletion showed no differences from an isogenic wild-type strain for any of the phenotypes tested. *Nup2*[–] strains mate with wild-type efficiency, and so a *nup2-4::URA3/nup2-4::URA3* diploid (JLX78) was constructed and used for the immunofluorescence experiments described in the preceding section. Examination of the *nup2* deletion strains by Nomarski microscopy failed to reveal any morphological abnormalities. Furthermore, no defects were detected in nuclear structure, as visualized by 4,6-diamidino-2-phenylindole staining of DNA or in the nuclear envelope as visualized by immunofluorescence of NPC antigens with the 306 MAb. The viability of strains deleted for *NUP2* contrasts with the inviability of null mutants of *NUP1* and *NSP1* (Hurt, 1988; Davis and Fink, 1990).

To test the effects of *NUP2* overexpression, we constructed a transcriptional fusion of the *NUP2* coding sequence to the *GAL1-10* promoter (plasmid pJON86) and introduced this construct into a wild-type strain. When *NUP2* overexpression was induced by addition of galactose to the media, the cells continued to grow but at an extremely reduced rate. The partial inhibition

of growth on overexpression of *NUP2* was also observed in strains bearing *NUP2* on 2μ plasmids. In contrast, overexpression of *NUP1* leads to complete cessation of growth (Davis and Fink, 1990). The failure of *NUP2* to inhibit growth as dramatically as *NUP1* upon overexpression could be due to the fact that *NUP2* levels are not as high as *NUP1* upon induction. Analysis of the level of *NUP2* expression on glucose and galactose media by Western blotting showed that the *NUP2* protein content was induced only 2- to 5-fold as compared with the >10-fold increase previously shown for the *GAL::NUP1* construct.

NUP2 Function is Required for Viability in Strains Carrying Truncations of *NUP1*

A series of genetic experiments was performed to identify functional overlap or redundancy between *NUP2* and the other yeast nucleoporins. The similarity in primary structure of *NUP2* to the other yeast genes suggested that segments of other nucleoporins might compensate for the absence of the *NUP2* gene product in *nup2*Δ strains. Strains carrying an amino terminal truncation, *nup1-8* (deleted for amino acids 4-141), or a carboxy-terminal truncation, *nup1-21* (deleted for amino acids 1042-1076), on centromere containing plasmids covering a *nup1* null mutation grow somewhat more slowly than strains carrying the full-length *NUP1* on a plasmid (Davis, unpublished observations). However, these same *nup1* truncation alleles are inviable in a *nup2*Δ background. This result was obtained by a "plasmid shuffle" (Boeke *et al.*, 1984) assay using strains harboring two plasmids; one containing a wild-type copy of the *NUP1* gene (*NUP1 URA3 CEN*) and the other, a *nup1* truncation (on a *CEN TRP1* vector). The chromosome contained a deletion of *nup1* marked with the *LEU2* gene (*nup1-2::LEU2*; Davis, unpublished data) and either a wild-type copy of *NUP2* (strain L4745) or a complete deletion of *NUP2* marked with *HIS3* (JLY119). These strains were grown on synthetic complete (SC) media lacking tryptophan but including uracil, which permits the growth of segregants that have lost the *NUP1 URA3* plasmid. Segregants that have lost *NUP1* could be identified by replica plating onto SC + 5-FOA, a medium that selects against strains that retain the *URA3 NUP1* plasmid.

Strains containing a functional *NUP2* could lose the wild-type *NUP1* plasmid when provided with either *NUP1* truncation mutant and hence papillated on the medium containing 5-FOA (Figure 7). These *NUP2 nup1* (truncation) strains grow more slowly than *NUP2 NUP1* strains but form colonies on standard medium. By contrast, strains deleted for *NUP2* cannot sustain growth with either *nup1* truncation, because they are unable to lose the *NUP1* wild-type plasmid and thus do not grow in the presence of 5-FOA. Therefore, strains bearing certain deletions of the amino and carboxy-terminal regions of *NUP1* require a functional *NUP2* gene.

Figure 7. Synthetic lethality between truncations of *NUP1* and *nup2 Δ* . *nup1-2::LEU2* yeast strains bearing the indicated *NUP1* allele on a *TRP1 CEN* plasmid, shown schematically, as well as wild-type *NUP1* on a *URA3 CEN* plasmid and the indicated allele of *NUP2* were patched onto synthetic complete medium lacking tryptophan, grown to confluency, and then replica plated to synthetic complete medium containing 5-FOA. Photo was taken at 48 h after transfer to 5-FOA media.

Either the Amino Terminus of NUP2 or an Amino Terminal Region of NUP1 is Required for Growth

Because cells require *NUP2* in some *nup1* mutant backgrounds, we were able to develop an assay to test the ability of various *NUP2* constructs to provide the *NUP2* function required in these *nup1* strains. We modified the plasmid shuffle strategy described above by constructing a *TRP1 CEN* plasmid bearing both wild-type *NUP2* and the *nup1-8* amino terminal truncation of *NUP1* (pJON139) and introducing it into the *nup1 Δ nup2 Δ* strain (JLY119) used previously. Upon loss of the wild-type *NUP1 URA3* plasmid, this construct must provide both the *NUP1* specific function, as well as the overlapping function of *NUP1* and *NUP2*. As predicted,

the *nup1-8 NUP2* plasmid conferred viability to the *nup1 Δ nup2 Δ* strain (Figure 8, top row). One limitation of this assay is the appearance of fast-growing colonies in patches of the negative control strain, which lacks active *NUP2* (Figure 8, bottom row). These suppressed strains are probably the result of recombination between the *NUP1* wild-type gene on the *URA3* plasmid and the mutant *nup1-8* gene on the second plasmid. The appearance of these colonies is distinguished from the growth observed in other patches by their low frequency and by their rapid rate of growth; therefore, even inefficient complementation can be detected by this assay.

We first used this assay to confirm that the modified version of *NUP2* bearing the HA epitope is capable of

Figure 8. Complementation of synthetic lethality by truncations of *NUP2*. A *nup1-2::LEU2*, *nup2-5::HIS3* strain bearing wild-type *NUP1* on a *URA3 CEN* plasmid (JLY119) was transformed with a second plasmid bearing the *nup1-8* amino terminal truncation and the indicated allele of *NUP2*. The plasmid shuffle assay was then performed as in Figure 7. Photo was taken after 4 d at 30°.

providing the function shared between NUP1 and NUP2. The ability of *NUP2::HA* to complement is evidence that the tagged protein used for immunofluorescence localization experiments is functional, as well as localized at the nuclear envelope (as shown in Figure 5).

To determine which domain(s) of *NUP2* are genetically overlapping with the *NUP1* amino terminal truncation mutant, we constructed three carboxy-terminal truncations, *nup2-9*, *nup2-10*, and *nup2-11*, in the *nup1-8 NUP2* plasmid. These constructs were made by introducing the HA epitope followed by a series of three stop codons into *NUP2* after amino acids 85, 174, and 555, respectively. Surprisingly, the *nup2-10* and *nup2-11* plasmids were capable of partially complementing the synthetic lethal phenotype in the plasmid shuffle assay (Figure 8). After loss of the *NUP1* wild-type plasmid, both *nup1-8 nup2-10* and *nup1-8 nup2-11* strains grow slowly compared with the *nup1-8* strain with the wild-type *NUP2* allele. In contrast, the most extensive deletion, *nup2-9*, was completely incapable of complementing the *nup1-8 nup2Δ* synthetic lethal phenotype. The partial complementation of *nup1-8 nup2Δ* by carboxy-terminal truncations of *NUP2* implies that the amino terminal region, which precedes the repeated domain of *NUP2*, is able to provide the essential activity necessary in strains lacking the amino-terminal 141 amino acids of *NUP1*.

The Amino Terminus of NUP2 is also Required for the Function of Amino Terminal Truncations of NSP1

Various mutations of *NSP1* and *NUP2* were tested in combination to determine whether these two genes also interact. A summary of these results is presented in Table 3. In strains carrying a functional copy of *NUP2*, the carboxy-terminal 217 amino acids of *NSP1* (C-*NSP1*) are sufficient to complement the function of a null *nsp1* mutant (Nehrbass *et al.*, 1990). To ask whether C-*NSP1* could suffice in the absence of *NUP2*, we examined the viability of the *nup2Δ* C-*NSP1* double mutant among spores arising from a diploid strain with the genotype *nsp1::URA3/NSP1*, *nup2-7::TRP1/NUP2* (JLX53), containing C-*NSP1* expressed from the *ADH* promoter on a *LEU2* *CEN* plasmid. No *nup2Δ* C-*NSP1* double mutant spores (Ura⁺ Trp⁺ Leu⁺) were ever recovered among the ascospore progeny (40 tetrads examined), whereas both single mutants (*nup2Δ NSP1* and *NUP2 C-NSP1*) appeared at the expected frequency. In a control cross using an isogenic strain bearing an *NSP1 LEU2 CEN* wild-type plasmid, ~50% of Ura⁺ Leu⁺ spores contained *nup2-7::TRP1* deletion, as expected. These data suggest that the amino terminal and repetitive domains of *NSP1* are dispensable only in strains carrying a functional *NUP2* gene. To define the region of *NSP1* that is required in a *nup2Δ* background, we performed another

Table 3. Growth of *NSP1 NUP2* double mutants

<i>NSP1</i> genotype	<i>NUP2</i> genotype	Ascospore growth
<i>NSP1</i>	<i>NUP2</i>	+++
<i>nsp1Δ</i> 173-605	<i>NUP2</i>	+++
<i>nsp1Δ</i> 173-605	<i>nup2Δ</i> ^a	+++
C- <i>NSP1</i>	<i>NUP2</i>	+++
C- <i>NSP1</i>	<i>nup2Δ</i>	—
C- <i>NSP1</i>	<i>nup2Δ</i> 175-720 ^b	+

+++ , wild-type growth; — , did not germinate.

^a *nup2-7::TRP1*, a precise replacement of the *NUP2* coding sequence with the *TRP1*.

^b *nup2-12::TRP1*, replacement of the internal *Apa* I fragment of *NUP2* with the *TRP1* gene. Adds the sequence EFHMLKstop after amino acid 172 of *NUP2*.

cross using a construct in which only the repetitive domain of *NSP1* (amino acids 173–605 deleted) (Nehrbass *et al.*, 1990) is deleted. In this case the double mutant (*nup2Δ nsp1Δ* 173-605) is viable but grows more slowly than either single mutant. This result indicates that the 172 N-terminal amino acids of *NSP1* must be vital for the shared function of *NSP1* and *NUP2* and, furthermore, that cells can tolerate the simultaneous loss of the repeated domains of *NSP1* and *NUP2*.

To test whether a similar relationship exists between the amino termini of *NUP2* and *NSP1* as was shown for *NUP2* and *NUP1*, a chromosomal truncation of *NUP2* (*nup2-12*, Δ175-720; pJON166 in MATERIALS AND METHODS), similar to the plasmid borne *nup2-10* was constructed and crossed to the C-*NSP1* strain. This cross was analyzed, and viable C-*NSP1 nup2-12* double mutants were recovered. Because this double mutant is viable, whereas the C-*NSP1 nup2Δ* mutant is lethal, the amino terminus of *NUP2* must provide the shared function with the amino terminus of *NSP1*.

The synthetic lethality between mutant alleles of *NSP1*, *NUP1*, and *NUP2* appears to be specific because mutations in other genes implicated in nuclear transport do not show any interactions with *nup2Δ*. Double mutants between the *nup2* deletion and *nsr1-1::HIS3*, a null allele of a putative nuclear localization sequence receptor (Lee *et al.*, 1991), were tested for viability. The *nsr1-1 nup2* double mutant grows at the same rate as the *nsr1-1 NUP2* strain, suggesting that there is no interaction between the two mutations. We also examined double mutants between *nup2Δ* and *rna1-1*, a temperature-sensitive allele of a gene encoding a cytoplasmic protein required for the export of mRNA from the nucleus (Hutchinson *et al.*, 1969; Hopper *et al.*, 1990). Again, the double mutant *nup2Δ rna1-1* fails to show any phenotype indicative of a *NUP2 RNA1* interaction.

DISCUSSION

We have isolated a gene encoding a new nucleoporin, NUP2 (95 kDa). Like the two previously identified yeast nucleoporins, NUP1 (130 kDa) (Davis and Fink, 1990) and NSP1 (100 kDa) (Nehrbass *et al.*, 1990), NUP2 is recognized by a panel of monoclonal antibodies that cross-react with nucleoporins from many different eukaryotes. Several lines of evidence suggest that NUP2 is a component of the nuclear pore complex. The patchy punctate staining of the nuclear envelope observed by indirect immunofluorescence of epitope tagged NUP2 protein is very similar to that shown for other NPC proteins in yeast (Davis and Fink, 1990; Nehrbass *et al.*, 1990). Furthermore, NUP2 and NSP1 are the only nuclear proteins that are recognized by MAb 414 (Aris and Blobel, 1989; Davis and Fink, 1990; this work), which has been shown to be specific for mammalian nuclear pore complexes by immunoelectron microscopy (Davis and Blobel, 1986). The three cloned yeast nucleoporins have a similar structure: each can be divided into three domains defined by a centrally located segment consisting of a loosely conserved nine amino acid repeating unit (the FSF repeated motif) that is separated by highly charged spacers of variable length. The sequence homology between NUP2, NUP1, and NSP1 is restricted to the central domain. Several other proteins associated with yeast nuclei are recognized by the anti-nucleoporin MAbs. In particular, there is a group of three proteins of 45–65 kDa (Davis and Fink, 1990). Why did our screen fail to identify any of these proteins? Of 13 immunoreactive clones, only NUP1 and NUP2 hybridized strongly to the degenerate NSP1 repeat oligonucleotide, suggesting that NSP1, NUP1, and NUP2 may be the only proteins in yeast with similar repetitive domains. However, we cannot rule out either that there are other nucleoporins with repetitive domains that were underrepresented in our library or that there is a class of nucleoporins that lacks the FSF repeat but still is recognized by the anti-nucleoporin MAbs.

NUP2, unlike NUP1 or NSP1, is not essential for viability because deletion of the entire coding sequence of NUP2 has no effect on growth. However, NUP2 is required in strains containing certain mutations in either NUP1 or NSP1. Small truncations from either end of NUP1 are viable in a strain with a functional NUP2 gene but are lethal in combination with a NUP2 deletion. Similarly, a strain expressing only the carboxy-terminal domain of NSP1 (a deletion of the amino-terminal and repeated domains) is viable with a functional NUP2 gene but is inviable in conjunction with a NUP2 deletion. This "synthetic lethality" among yeast nucleoporins provides the first evidence for functional interaction between these NPC components. These relationships suggest that the nucleoporins have similar activities or that they interact physically. However, we also have found that NUP2 protein in high dosage is unable to

supply the essential functions missing in *nup1Δ* or *nsp1Δ* strains, which underscores the fact that members of the family also must have unique activities. In addition, the inviability of these double mutants also rules out the existence of another protein that is completely equivalent to NUP2, although there might be more proteins that are partially redundant or interact in a similar mode. In light of the genetic interactions identified between NUP2 and both NSP1 and NUP1, it would be informative to construct double mutations between NUP1 and NSP1, which might provide further clues as to which regions of the nucleoporins specify unique functions and which regions are genetically overlapping.

Our data suggest an important role for the amino-terminal domain of each of the three nucleoporin genes. Amino-terminal truncations of NUP1 have progressively more severe phenotypes, and truncations with mild phenotypes are exacerbated by deletion of NUP2. Furthermore, although the amino terminus of NSP1 is dispensable in strains carrying a functional NUP2 gene, it becomes essential in the absence of NUP2 protein. What is remarkable is that the amino-terminal domain of NUP2 is, expressed by itself, able to confer viability to strains carrying amino-terminal truncations of either NUP1 or NSP1. These data suggest that, in a genetic sense, the amino-terminal domains of these three proteins are functionally redundant; in other words, either one polypeptide or the other is required. Strikingly, their amino acid sequences contain no significant similarities. Despite the fact that we have shown an important role for the amino terminus of all three yeast nucleoporins, the only mammalian nucleoporin cloned, p62, which has extensive homology to NSP1, does not have an analogous domain amino terminal to its repeated domain, implying there are functionally significant differences between the nucleoporins in yeast and their metazoan counterparts.

It is likely that the central repeated domain, common to all three yeast nucleoporins, has a similar function in each protein. The conservation of the nucleoporin repeated motif in widely divergent species suggests its importance in NPC function. However, we have been unable to create a situation where it has an essential role *in vivo* for any of the yeast nucleoporins. Deletion of the repeats individually or simultaneously from NSP1 or from NUP2 does not affect viability. One explanation for the dispensability of the repeats could be that the role of this domain is critical, but it can be supplied by any one of the repeat bearing nucleoporins. If this were the case, then it implies that the repeated domain can function independently of any of the specific functions of the termini of these proteins. The fact that the terminal segments of NUP2 and NSP1 are active when expressed by themselves further suggests that the repeated domains could not be involved directly in assembling or positioning the terminal domains into their active form. Furthermore, this model predicts that si-

multaneous deletion of the central domain from each repeat bearing nucleoporin would be lethal.

We propose that the synthetic lethality in these double mutant strains (*nsp1nup2* or *nup1nup2*) reflects an underlying similarity in action among these proteins. Two general models have been suggested to explain synthetic lethality. One is that it reflects protein-protein interactions within a multisubunit complex: a complex may function if one constituent is defective but may not function if multiple constituents are defective. For example, temperature-sensitive mutations in *SEC17* and *SEC18*, yeast homologs of the mammalian proteins NSF and SNAP, known to be part of a protein complex involved in membrane fusion, are lethal in combination (Clary *et al.*, 1990; Kaiser and Schekman, 1990). An alternative explanation for synthetic lethality is that two or more proteins have redundant activities that carry out some essential function. Loss of function of one protein can be compensated by the activity of the other; when neither is available the cell, unable to carry out the necessary activity, is inviable. Most instances of this type of relationship involve families of highly similar genes, such as tubulin (Huffaker *et al.*, 1987) or protein kinases (Toda *et al.*, 1987). Our genetic experiments cannot distinguish between these two possibilities. Although the yeast genes are superficially similar in sequence organization, the sequence similarity is restricted to the central domains. Moreover, it is the nonhomologous terminal domains that exhibit functional overlap. The partial sequence similarity and partial redundancy of function is reminiscent of the yeast G1 cyclin family (Richardson *et al.*, 1989). Perhaps, like cyclins, nucleoporins operate as units of a modular system, where each of these molecules physically interacts with the same complement of accessory proteins, and complexes including different sets of nucleoporins serve similar but distinct roles in NPC function. Biochemical experiments underway in our laboratories could provide evidence for the postulated complexes and their function in nuclear transport.

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