Genetic and Physical Interactions between Srp1p and Nuclear Pore Complex Proteins Nup1p and Nup2p

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Abstract. Nup1p is a yeast nuclear pore complex protein (nucleoporin) required for nuclear protein import, mRNA export and maintenance of normal nuclear architecture. We have used a genetic approach to identify other proteins that interact functionally with Nup1p. Here we describe the isolation of seventeen mutants that confer a requirement for Nup1p in a background in which this protein is normally not essential. Some of the mutants require wild-type Nup1p, while others are viable in combination with specific *nup1* alleles. Several of the mutants show nonallelic noncomplementation, suggesting that the products may be part of a hetero-oligomeric complex. One is allelic to *srp1* which, although it was identified in an unrelated screen, was shown to encode a protein that is localized to the nuclear envelope (Yano, R., M. Oakes, M. Yamaghishi, J. A. Dodd, and M. Nomura. 1992. *Mol. Cell. Biol.* 12:5640–5651). We have used immunoprecipitation and fusion protein precipitation to show that Srp1p forms distinct complexes with both Nup1p and the related nucleoporin Nup2p, indicating that Srp1p is a component of the nuclear pore complex. The distant sequence similarity between Srp1p and the β-catenin/desmoplakin family, coupled with the altered structure of the nuclear envelope in *nup1* mutants, suggests that Srp1p may function in attachment of the nuclear pore complex to an underlying nuclear skeleton.

THE nuclear pore complex (NPC)[1] is a hetero-oligomeric structure that forms a large channel through the nuclear envelope. It is the only known conduit for export of newly synthesized RNA from the nucleus to the cytoplasm, and for import of nuclear proteins (for review see Forbes, 1992; Silver, 1991; Davis, 1992). A detailed understanding of the mechanisms by which the NPC controls macromolecular traffic, and indeed whether it has other functions as well, has been hindered by the complexity of its structure. It is estimated to contain 100–200 different polypeptides and to have a mass of about 112 megadaltons (Reichelt et al., 1990). The core is composed of an eightfold symmetrical spoke-ring structure, surrounding a central plug or "transporter" (Unwin and Milligan, 1982; Akey, 1989; Hinshaw et al., 1992; Akey and Radermacher, 1993). Using other methods of sample preparation, several groups have reported the presence of a basket extending from the nuclear ring 50–100 nm into the nucleoplasm, as well as filaments protruding out from the cytoplasmic ring (Ris, 1991; Jarnik and Aebi, 1991; Goldberg and Allen, 1992). Scanning EM of *Triturus* oocytes has also revealed the presence of a hexagonal filamentous lattice, distinct from the nuclear lamina, in which the annuli of the baskets are embedded (Goldberg and Allen, 1992). The composition of this lattice is unknown, and it remains to be determined whether it is a ubiquitous feature of nuclear envelope structure. These recent observations suggest that, in addition to regulating nuclear transport, the NPC may serve to attach the nuclear envelope to an underlying nuclear skeleton, and perhaps to cytoskeletal elements as well.

A small number of NPC proteins (nucleoporins) have been characterized to date. Among them is a family of related polypeptides conserved from yeast to vertebrates (Davis and Blobel, 1986, 1987; Snow et al., 1987; Aris and Blobel, 1989; Davis and Fink, 1990). These proteins have amino acid similarity within a domain containing degenerate repeats, and the mammalian proteins at least are modified by the addition of O-linked *N*-acetylglucosamine (Davis and Blobel, 1987; Holt et al., 1987; Hanover et al., 1987). Yeast genes *NUP1*, *NUP2*, and *NSP1*, and vertebrate genes p62 and nup153, form one subfamily (Davis and Fink, 1990; Nehrbass et al., 1990; Starr et al., 1990; Carmo-Fonseca et al., 1991; Cordes et al., 1991; Sukegawa and Blobel, 1992; Loeb et al., 1993). Each has a domain consisting of degenerate XFXFG repeats that is recognized by mAbs 306 and 414. The carboxy-terminal domain of *NUP1* and nup153, and the

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1. *Abbreviations used in this paper*: CPRG, chlorophenolred-β-D-galactopyranoside; 5FOA, 5-fluororotic acid; GST, glutathione-S-transferase: NPC, nuclear pore complex; WGA, wheat germ agglutinin; X-gal, 5-brom-4-chlor-3-indolyl-β-D-galactopyranoside.

amino-terminal domain of *NSP1* are also distantly related to one another, and to the GLFG repeat domain that characterizes the other subset: *NUP49*, *NUP100*, and *NUP116* (Wente et al., 1992; Wimmer et al., 1992). This domain is recognized by mAb 192, and is also present in another vertebrate nucleoporin, p145 (Hallberg et al., 1993). Unlike the others, p145 is an integral membrane protein.

The exact localization of each of these proteins within the NPC is not clear. Snow et al. (1987) and Sukegawa and Blobel (1992) showed that antibodies specific for nup153 stain only the nucleoplasmic side of the NPC. This was confirmed more recently in studies showing that anti-p153 antibodies stain the distal ring of the nuclear basket (see Pante and Aebi, 1993). Interestingly, this protein has four zinc finger motifs and binds to DNA in vitro (Sukegawa and Blobel, 1992), raising the possibility that the annular ring contacts (and perhaps organizes) chromatin in the vicinity of the NPC. The annular ring is also the predominant structure stained by the lectin wheat germ agglutinin (WGA), suggesting that all of the O-linked NPC glycoproteins are localized there (Pante and Aebi, 1993). However, studies using different preparations have found WGA staining in the "central transporter" (Akey and Goldfarb, 1989). Furthermore, Snow and co-workers (1987) found that some mAbs decorated both nuclear and cytoplasmic sides, suggesting at least two separate locations.

In vertebrates, RNA export and the translocation step of protein import can be blocked either by monoclonal antibodies to the O-linked NPC glycoproteins (Featherstone et al., 1988; Dabauvalle et al., 1988) or by WGA (Dabauvalle et al., 1988; Finlay et al., 1987; Yoneda et al., 1987; Adam and Gerace, 1991). Nuclei reconstituted from egg extracts depleted of all or a subset of WGA-binding proteins are incapable of docking import substrates at the NPC (Finlay and Forbes, 1990; Finlay et al., 1991). Phenotypic analysis of yeast mutants further supports a role for these proteins in transport. Mutvei et al. (1992) found that depletion of *NSP1* resulted in cytoplasmic accumulation of nuclear-targeted proteins. In work to be reported elsewhere (Bogerd, A. M., J. A. Hoffman, D. C. Amberg, G. R. Fink, and L. I. Davis, manuscript submitted for publication), we have shown that temperature-sensitive mutations in *NUP1* cause pleiotropic defects upon shift to the nonpermissive temperature. Import of an H2B-β-galactosidase fusion protein stops, and poly(A) containing RNA accumulates in the nucleus, suggesting a defect in mRNA export. Moreover, these mutants are inviable in combination with mutations in *RNA1* (Bogerd, A. M., J. A. Hoffman, D. C. Amberg, G. R. Fink, and L. I. Davis, manuscript submitted for publication), which encodes a cytoplasmic protein required for RNA export (Shiokawa and Pogo, 1974; Hopper et al., 1990; Amberg et al., 1992). Thus these proteins appear to play a critical role in nucleo-

Table I. Yeast Strains Used in This Study

Strain	Genotype	Source
9933-13A	MATa *his3Δ200 leu2-3,112 ura3-52 trp1Δ1*	Fink collection
L3852	MATα *his3Δ200 lys2Δ201 leu2-3,112 trp1Δ1 SNP1*	Fink collection
L4745	MATα *trp1-101 ura3-52 leu2-3,112 his3Δ200 nup1-2::LEU2 snp1* (p2487)	Fink collection
LDY402	MATα *trp1-101 his3Δ200 leu2-3,112 nup1-2::LEU2 nup2-7::TRP1* (pLDB18)	This study
LDY176	*MATa his3Δ200 leu2 ura3-52 trp1Δ289 nup1-2::LEU2 snp1* {pBP2487}	Bogerd et al., manuscript in preparation
KBY51	*MATa ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY52	*MATα ade2-101 ade3 trp1Δ1 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY36	*MATa nle1/srp1 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY4	*MATα nle2 ade2-101 ade3 trp1Δ1 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY11	*MATa nle3 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY40	*MATa nle4 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY18	*MATa nle5 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY10	*Mata nle6 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY05	*MATα nle7 ade2-101 ade3 trp1Δ1 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY08	*MATα nle8 ade2-101 ade3 trp1Δ1 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY32	*MATa nle9 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY12	*MATa nle10 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY13	*MATa nle11 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY16	*MATa nle12 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY19	*MATa nle13 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY20	*MATa nle14 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY22	*MATa nle15 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MKY25	*MATa nle16 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
MBY35	*MATα nle17 ade2-101 ade3 trp1Δ1 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY44	*MATa nle1/srp1 ade2-101 ade3 trp1Δ1 lys2-801 ura3-52 nup1-2::LEU2 SNP1* (pLDB73)	This study
KBY70	*MATα nle1/srp1 ade2-101 his3Δ200 lys2-801 nup1-2::LEU2 SNP1 SRP1::HIS3* (pLDB73)	This study
BJ926	*MATa/α pre1-126 pep4-3 prb1-1122 can1-11 gal2*	B. Jones
LDY74	*MATα ura3-52 leu2-3,-112 trp1-289 can1 cyh2 GAL10::NUP1::LEU2*	This study
Y526	*MATa ade2-101 his3Δ200 leu2-3,112 trp1-901 gal4Δ512 gal80Δ338 URA3::GAL1::lacZ*	S. Fields
1938	*MATa arg9 leu2 TWL-o*	Reed Wickner

cytoplasmic transport, although their exact function(s) remain unclear.

Combinatorial analysis of yeast nucleoporin mutants has revealed that *NUP2*, which is normally dispensable, becomes essential when combined with truncations of *NUP1* or *NSP1* (Loeb et al., 1993). *NSP1* truncations are also lethal in combination with deletions of *NUP116* and *NUP49* (Wimmer et al., 1992). Such synthetic lethality suggests functional and/or physical association between the two proteins. Physical association between Nsplp and Nup49p has recently been demonstrated. These two proteins form a complex with Nup54p and a structurally unrelated protein called Nic96p (Grandi et al., 1993).

The observation that *nup1* mutants show synthetic lethality with mutations in genes encoding two other proteins implicated in NPC function (*NUP2* and *RNA1*) suggested that a screen for new mutants that are lethal in combination with *nup1* might identify novel nuclear pore complex proteins, as well as nuclear and cytoplasmic proteins that may be involved in NPC function. Here we describe the results of such a screen. 17 mutants have been identified. One of these is allelic to *srp1*, a gene previously identified as a suppressor of mutants defective in RNA polymerase I (Yano et al., 1992). We also show that Srplp forms distinct complexes with both Nuplp and Nup2p.

Materials and Methods

Reagents

Enzymes for molecular biology were purchased from Boehringer-Mannheim Biochemicals (Indianapolis, IN), Pharmacia (Piscataway, NJ), and New England Biolabs (Beverly, MA). Lyticase was purchased from Enzogenetics (Corvallis, OR). Protein A–Sepharose and glutathione-Sepharose were purchased from Pharmacia. 5-fluororotic acid (5FOA) and 5-brom-4-chlor-3-indolyl-β-D-galactopyranoside (X-gal) were obtained through the Genetics Society of America consortium (Bethesda, MD). Antiserum to Srplp was generously provided by Dr. Masayasu Nomura (University of California, Irvine, CA). The yeast genomic library carried in pRS200 (Connelly, M., and P. Hieter, unpublished data) was furnished by Dr. Phil Hieter (Johns Hopkins University, Baltimore, MD), as were all of the pRS vectors (Sikorski and Hieter, 1989). Antibody 12CA5 was obtained from the Harvard Cell Culture Facility (Cambridge, MA). pGAD424 and pGBT9 were furnished by Dr. Stan Fields (State University of New York, Stony Brook, NY).

Strains and Microbial Techniques

The yeast strains and plasmids used are listed in Tables I and II. DNA clon-

Table II. Plasmids Used in This Study

Strain	Markers	Comments	Source
p2487	*CEN URA3 NUP1*	*NUP1* wild-type in pRS316*	Loeb et al., 1993
pLDB73	*CEN URA3 ADE3 NUP1*	BamHI NheI *ADE3* fragment blunt-ended into BamHI of p2487	This study
pLDB18	*CEN HIS3 NUP1^{HA}*	*NUP1^{HA}* fragment (XhoI BamHI) into pRS313	This study
pLDB107	*CEN TRP1 NUP1*	*NUP1* fragment (XhoI BamHI) into pRS314	This study
pLDB26	*CEN TRP1 nup1-8*	Truncation of residues 4-141 from *NUP1*	Bogerd et al., in prep.
pLDB33	*CEN TRP1 nup1-15*	Truncation of residues 4-191 from *NUP1*	Bogerd et al., in prep.
pLDB34	*CEN TRP1 nup1-21*	Truncation of residues 1042-1076 from *NUP1*	Bogerd et al., in prep.
pKBB6	*CEN TRP1 NLE/SRP1*	8.5 kb fragment containing *NLE1/SRP1* from pRS200 library‡	This study
pKBB7	*CEN TRP1 NLE1/SRP1*	10.6 kb fragment containing NLE1/SRP1 from pRS200 library	This study
pKBB8	*CEN TRP1 NLE1/SRP1*	13 kb fragment containing *NLE1/SRP1* from pRS200 library	This study
pKBB9	*CEN TRP1 nle1/srp1*	pKBB6 digested with EcoRI and religated to remove 3.6 kb from insert	This study
pKBB10	*CEN TRP1 nle1/srp*	3.0 kb PstI fragment cloned from pKBB9 into pRS314	This study
pKBB11	*CEN TRP1 NLE1/SRP1*	HpaI and BglII fragment from pKBB6 cloned into SmaI BamHI site in pRS314	This study
pKBB13	*HIS3 NLE1/SRP1*	ClaI NotI fragment from pKBB11 cloned into ClaI NotI of pRS303	This study
pGBT9	*2μLEU2 GAL4*-AD	*GAL4* DNA-binding domain for two-hybrid system	Fields and Song, 1989
pGAD424	*2μ TRP1 GAL4*-BD	*GAL4* activation domain for two-hybrid system	Fields and Song, 1989
pSWB11	*2μLEU2 GAL4*-AD *nup1*	*nup1* residues 655-1076 in pGBT9; see Materials and Methods	This study
pSWB17	*2μTRP1 GAL4*-BD *NLE1/SRP1*	*NLE1/SRP1* in pGAD424; see Materials and Methods	This study
pLDB17	*LEU2 GAL10::NUP1^{HA}*	*GAL10* promoter inserted at SnaB1 site of *NUP1^{HA}* in pRS305	This study
Bacterial	Description		
pB2337	*nup1-2::LEU2*		Loeb et al., 1993
pJON13	*nup2-7::TRP1*		Loeb et al., 1993
pGEX-2TK	Vector for GST fusion constructs		Kaelin et al., 1992
pSWB1	*NUP1* residues 5-385 in pGEX-2TK; see Materials and Methods		This study
pSWB5	*NUP1* residues 432-816 in pGEX-2TK; see Materials and Methods		This study
pSWB6	*NUP1* residues 778-1076 in pGEX-2TK; see Materials and Methods		This study

* All pRS vectors were obtained from P. Hieter (Sikorski and Hieter, 1989)
‡ Connelly and Hieter, unpublished data.

ing was performed using standard techniques outlined in Sambrook et al., 1989). Yeast cell culture, media preparation, and genetic manipulations were performed essentially according to Sherman et al. (1986). Yeast shuttle plasmids and linear fragments were introduced into yeast by lithium acetate transformation (Ito et al., 1983). Selection against Ura^+ strains was accomplished by culture on solid synthetic media containing 1 mg/ml 5FOA (Boeke et al, 1987). Sectoring assays were performed on solid synthetic media containing 25% of the normal amount of adenine and histidine supplements (SC_{sec}).

Identification of SNP1

When a diploid obtained by mating strains L3852 and 9933-13A was disrupted by insertion of *nupl-2::LEU2*, viable Leu^+ spores were recovered after sporulation and tetrad dissection. Of the 93 tetrads examined, the ratio of those containing 2:2, 3:1, and 4:0 viable segregants was ~1:4:1, suggesting that the diploid was also heterozygous for an unlinked bypass suppressor segregating as a single gene. This was confirmed by crossing a Leu^+ segregant to L4745 (*nupl-2::LEU2* carrying a *NUP1 URA3* plasmid), a strain that requires plasmid borne *NUP1* gene for viability. Spores obtained after tetrad dissection were tested for their ability to grow in the absence of the plasmid-borne *NUP1* gene by plating on media containing 5FOA. Viability on 5FOA segregated 2:2, confirming that the suppressor is encoded by a single gene. Backcrosses to each parent identified L3852 as the strain containing the suppressor, because all haploid segregants from this cross were viable on 5FOA. We have designated this gene *S*uppressor of *NuP* (*SNP1*).

Isolation of Mutants

The colony sectoring assay used to identify mutants synthetically lethal with *nupl* has been previously described (Bender and Pringle, 1991). Chemical mutagenesis was performed on strains KBY51 and KBY52 with ethylmethane sulfonate using standard procedures (Lawrence, 1991) to produce 10% survival. UV irradiation to 40% survival was performed in a parallel screen, using 150 mJ of energy from a Stratalinker (Stratagene Corp., La Jolla, CA). Cells were plated on 150 mm^2 petri dishes at a density of 1,200 colonies each. After 5 d of incubation at 25°C, 1,500 nonsectoring (Sec^-) colonies were picked and scored for viability on 5FOA at 25°C. Of these, 150 were Sec^- and FOA^s. 35 of these were incapable of growth on a nonfermentable carbon source and were discarded. The remaining mutants were each transformed with a plasmid containing a wild-type *NUP1* gene and a different selectable marker (pLDB18 into strain KBY51, or pLDB107 into strain KBY52). Those that regained the ability to sector after transformation, indicating that the new mutation conferred a requirement for *NUP1*, were backcrossed to the parent strain. 17 segregated as single mutations. Strains in which the mutant phenotype failed to show 2:2 segregation were not studied further. All of the remaining mutants were recessive.

Cloning of NLE1

Cold-sensitive strain KBY44 was transformed with a yeast genomic library in pRS200 (Connelly, M., and F. Hieter, unpublished data), and transformants were grown at 30°C for 4 d. Colonies were replica plated and incubated at 16°C. Viable colonies were then scored on 5FOA and SC_{sec} at 30°C to identify those that had also become $5FOA^r$ Sec^+. Complementing plasmids were rescued as described by Strathern and Higgins (1991), and transformed into *Escherichia coli* strain DH5α. Plasmid DNA was isolated and used to retransform KBY44. All plasmids retained the ability to rescue both the cs and the Sec^- phenotypes. These were restriction mapped and subcloned using standard procedures. Double-stranded dideoxy sequencing from the EcoRI site of pKBB10 was performed using manufacturer's instructions (United States Biochemical Corp., Cleveland, OH).

Two-hybrid Analysis

A *NUP1* fragment containing carboxy-terminal amino acid residues 655 to 1,076 was amplified by PCR, using oligonucleotides 5'-GCGCCGGAATTCCCCTCCTTTACGTTTGCGTCTTCAAAAACCTCAC-3' and 5'-GGGCGCGGATCCACCAGGTATCCATTATCTCGCCTGTACCCG-3', and cloned into EcoRI/BamHI sites of pGBT9 (Fields and Song, 1989), to create an in frame fusion with the Gal4 DNA-binding ($GAL4_{DB}$) domain, generating pSWB11. An in-frame fusion between the Gal4 activation domain ($GAL4_{AD}$) and amino acid residues 30 to 542 of Srplp was constructed by cloning a PstI fragment from pKBB8 into pGAD424, to generate pSWB17.

Yeast strain Y526 was co-transformed with the various pGBT9 and pGAD424 constructs. Transformants were tested for β-galactosidase expression by scoring for blue colonies on SSX media (Chien et al., 1991). To quantitate expression, liquid cultures were induced and assayed colorimetrically using chlorophenolred-β-D-galactopyranoside (CPRG) as described (Bogerd et al., 1993).

Metabolic Labeling and Cell Fractionation

Yeast cells in early log phase were labeled as described in Kolodziej et al. (1991), using 5 mCi of [^{35}S] Trans label (ICN Radiochemicals, Irvine, CA) to label 80 OD_{600} of cells. Cells were labeled for 2 h, and spheroplasted as described by Kalinich and Douglas (1989). Spheroplasts were lysed by resuspension in 10 ml ice cold 20 mM Hepes-HCl, pH 7.5, 5 mM $MgCl_2$, and protease inhibitors, following by vortexing and incubation on ice for 5 min. Lysates were centrifuged at 9,000 rpm in an SA600 rotor for 45 min at 4°C. The supernatant (S) was decanted and the pellet was resuspended in 5 ml ice cold 20 mM Hepes-HCl, pH 7.4, 5 mM $MgCl_2$; 1 M NaCl, and protease inhibitors, incubated on ice 10 min and centrifuged at 9,000 rpm for 45 min. The supernatant (P) was collected. Unlabeled extracts were fractionated in an identical manner.

GST Fusion Construction

Each of the three domains of *NUP1* was cloned into pGEX-2TK to produce glutathione-S-transferase (GST) fusion proteins. A fragment encoding amino acids 5 to 385 was generated by PCR amplification using oligonucleotides: 5'-CCGGGATCCACTTCTGTGATGTCTCCACG-3' and 5'-CCCGAATTCCTAGAATGATAAGGTAGGCGCCTCGGCAGTT-3', and cloned into the BamHI/EcoRI sites of pGEX-2TK, to produce an in-frame fusion with GST (pSWB1). The same protocol was used to amplify and clone the central repetitive domain (amino acids 432 to 816) using oligonucleotides of sequence: 5'-CCGGGATCCAGTGAAAATCATACAGAATCAGACGCG-3' and 5'-CCGGAATTCCTAGTTTGCTGAGGCATTGGTACCATTGG-3' to generate pSWB5, and the carboxy-terminal domain (residues 778 to 1076) with oligonucleotides: 5'-CCGGGATCCTCAACGCCGATTCCTGTCTTGGG-3' and 5'-CCCGAATTCCTACAACACAATACCTAATTACATAACCG-3' to generate pSWB6.

GST-Nuplp Precipitation and Immunoprecipitation

Expression of GST fusion proteins in *E. coli* and preparation of cell lysates were performed as described (Smith and Johnson, 1988). Lysates were incubated with glutathione-Sepharose at room temperature for 15 min in ELB (0.25 M NaCl, 0.05 M Hepes, pH 7.0, 5 mM EDTA, 0.5 mM DTT, 0.1% Tween 20), and the beads containing adsorbed fusion protein were washed three times. Yeast extracts (see above) were precleared by incubation with GST-Sepharose in ELB plus 1% nonfat dry milk for 1 h at 4°C. 100 μl of unlabeled extract or 100-200 μl of labeled extract (2.5×10^5 cpm) were used for each experiment. Precleared extracts were incubated with GST-Nuplp-Sepharose for 1 h at 4°C, after which the beads were washed five times with ELB. Bound protein was eluted in SDS sample buffer and subjected to SDS-PAGE. Labeled proteins were visualized by autoradiography. Unlabeled proteins were electrophoretically transferred to nitrocellulose. Blots were probed as previously described (Davis and Fink, 1990), except that BSA was replaced with 4% nonfat dry milk, and bound antibody was detected using the ECL detection system (Amersham Corp., Arlington Heights, IL).

For immunoprecipitation, anti-Srplp antibody was adsorbed to protein A-Sepharose by incubation in ELB plus 1% nonfat dry milk for 1 h at 4°C, after which the beads were washed two times in ELB. mAb 12CA5 was covalently coupled to protein A-Sepharose by cross-linking with dimethylpimelimidate, as described (Harlow and Lane, 1988). In both cases beads were then incubated with yeast extracts and processed as described above.

Results

Isolation of Mutants That Are Lethal in a ΔNUP1 Strain

Genetic crosses have previously identified two mutants exhibiting synthetic lethality with *nupl*; *rnal-1* (Bogerd, A. M., J. A. Hoffman, D. C. Amberg, G. R. Fink, and L. I. Davis, manuscript submitted for publication), a mutant that is defective for RNA processing and export (Hopper et al., 1978; Hutchinson et al., 1969; Shiokawa and Pogo, 1974); and a deletion of *NUP2*, which encodes a nucleoporin that is structurally related to *NUP1* and is normally nonessential

(Loeb et al., 1993). Because of the likelihood that these synthetic phenotypes reflect functional interactions in both cases, we initiated a screen to identify new mutants that show synthetic lethality with *nup1*.

Disruption of *NUP1* is lethal in many strain backgrounds (Davis and Fink, 1990; and unpublished results). However, we have identified a naturally occurring bypass suppressor of Δ*NUP1* (*SNP1*) that segregates as a single gene (see Materials and Methods). *nup1-2::LEU2 SNP1* strains grow much more slowly than wild-type, but exhibit no detectable defects in protein import or RNA export, both of which are phenotypes characteristic of compromised Nup1p function (Bogerd, A. M., J. A. Hoffman, D. C. Amberg, G. R. Fink, and L. I. Davis, manuscript submitted for publication). We used the *nup1::LEU2 SNP1* strain as the background for the synthetic lethal screen for two reasons. First, we would expect to obtain *snp1* mutants using this approach (assuming that *SNP1* represents a gain of function allele), thus enabling us to clone the gene. Second, because copy number has a substantial effect on the ability of all our existing *nup1* alleles to support growth in an *snp1* background (Bogerd, A. M., J. A. Hoffman, D. C. Amberg, G. R. Fink, and L. I. Davis, manuscript submitted for publication), any cellular mutation lowering expression of *nup1* could cause a synthetic phenotype with one of these alleles.

The screen was designed using an *ade2 ade3* colony-sectoring assay (Koshland et al., 1985, Fig. 1). The starting strains (KBY52: *MATα, nup1::LEU2 SNP1 ade2 ade3 ura3 trp1 lys2* or KBY51: *MATa, nup1::LEU2 SNP1 ade2 ade3 ura3 his3 lys2*) were transformed with pLDB73, a *CEN*-based plasmid containing a wild-type *NUP1* gene, as well as functional *URA3* and *ADE3* markers. Because these strains are capable of growth in the absence of *NUP1*, colonies will sector from red to white when grown on nonselective medium, due to loss of the plasmid-borne *ADE3* gene. Mutations in genes that are inviable in combination with a *NUP1* disruption will impose a selection on the plasmid. Colonies harboring such mutations will be nonsectoring (Sec$^-$) and sensitive to the addition of 5FOA to the medium (5FOA^s).

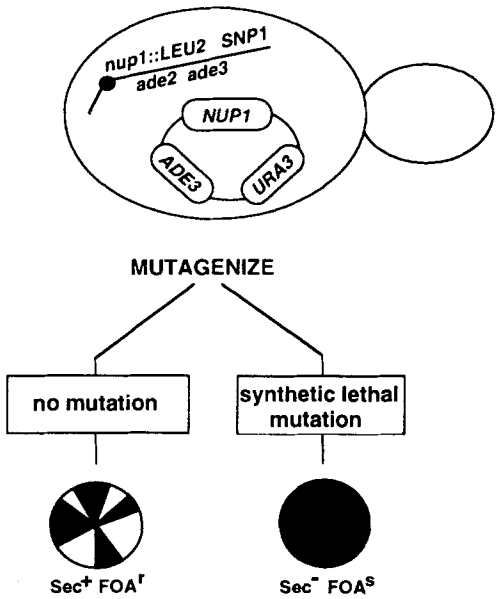

Figure 1. Strategy for isolating mutants synthetically lethal with *nup1*. The starting strains (KBY51 and 52) carry a *nup1-2::LEU2* deletion, and a suppressor that partially bypasses Nup1p function (*SNP1*). They are also *ade2 ade3*, and thus lack the red pigment synthesized in *ade2 ADE3* strains. The wild-type *NUP1* gene was introduced on a plasmid that also carries *ADE3* and *URA3* markers. Cells that retain the plasmid are Ura$^+$, 5-FOA^s, and red, whereas those that have lost the plasmid are Ura$^-$, 5-FOA^r, and white. Chromosomal mutations that render *NUP1* essential for viability confer a selection on the plasmid, and produce colonies that are FOA^s and do not sector from red to white (*Sec*$^-$).

Mutants were isolated and characterized as described in Materials and Methods. 17 mutants were identified that depended on wild-type *NUP1* for viability, and in which the Sec$^-$ 5FOA^s phenotypes segregated as a single mutation in backcrosses. We designated these *nle* (*n*up1 *l*ethal) *1* through *17*. Several of the mutants also exhibited conditional phenotypes; *nle2*, *4*, and *5* were incapable of growth (and *nle6* grew very poorly) at 36°C, and *nle1* was cold sensitive. In all cases the conditional phenotype segregated with synthetic lethality.

To determine the number of genes identified by this screen, each of the mutants was crossed to all those of the opposite mating type and diploids were selected by complementation of auxotrophic markers. Diploids were scored on 5FOA to determine complementation of the 5FOA^s phenotype (Table III). Although a number of the mutants failed to complement one another, they did not fall into distinguishable complementation groups. Sporulation and tetrad dissection of each noncomplementing diploid revealed that, in all but one case, the mutations segregated independently. Thus, many of the mutants in this collection exhibit nonallelic (or unlinked) noncomplementation.

Mutants *nle3* and *nle17* were the only two that showed tight linkage. They also showed identical allele specificity with *nup1* mutants, as discussed below. Furthermore, a DNA fragment isolated by complementation of *nle3* was also capable of rescuing *nle17*, suggesting that *nle3* and *nle17* are allelic. Preliminary sequence analysis of the complementing fragment suggests that *NLE3* has significant similarity throughout the coding region to the vertebrate nucleoporin nup153 (Kenna, M., J. Reilly, and L. Davis, unpublished data).

Allele Specificity

In studies to be reported elsewhere (Bogerd, A. M., J. A.

Table III. Complementation of nle Mutants

	nle2^{ts}	*nle7*	*nle8*	*nle17*	*NLE*
nle1^{sc}	−	+	−	−	+
nle3	+	−	+	−	+
nle4^{ts}	+	+	+	+	+
nle5^{ts}	+	+	+	+	+
nle6^{ts}	+	+	+	+	+
nle9	+	−	−	−	+
nle10-nle16	+	+	+	+	+
NLE	+	+	+	+	+

MATα strains are listed horizontally, *MATa* strains vertically. *nle3* and *nle17* mutations are linked. *NLE* strains are KBY51 (*MATa*) and KBY52 (*MATα*). Diploids were selected on SC -TRP -His. Growth of diploids on 5-FOA is indicated.

Hoffman, D. C. Amberg, G. R. Fink, and L. I. Davis, submitted), we have shown that deletion of the first 212 amino acids of Nuplp is lethal, and leads to mislocalization of the protein to the cytoplasm. Smaller deletion mutants are less tightly associated with the nuclear fraction than wild-type Nuplp, and strains carrying them grow at about half the rate of wild-type. Truncation of the carboxy-terminal domain does not affect the localization of the mutant protein, but has a severe effect on growth. These results suggest that the amino-terminal domain is required for localization of Nuplp to the NPC, whereas the carboxy-terminal domain is required for its function within the NPC. We thus used these alleles of *NUP1* (summarized in Fig. 2 *a*) to further characterize the collection of synthetic lethal mutants. Plasmids harboring functional amino-terminal (*nupl-8*, Δ 4-141 or *nupl-15*, Δ 4-191) or carboxy-terminal (*nupl-21*, Δ 1,042-1,076) truncations were transformed into each mutant, and their ability to support growth in the absence of wild-type *NUP1* was assayed by plating on 5FOA (Fig. 2 *b*). The mutants fell into four classes. Seven of them were not complemented by any of the truncations (class IV), whereas three were complemented by all of the *nupl* mutants (class I). Seven others showed allele specificity. Four of these were lethal in combination with the carboxy-terminal truncation mutant, *nupl-21*, but were complemented by *nupl-8*, and *nupl-15* (class II). In three cases (including allelic mutants *nle3* and *nlel7*), sectoring with *nupl-15* was very much reduced, whereas that with *nupl-8* was normal (class III).

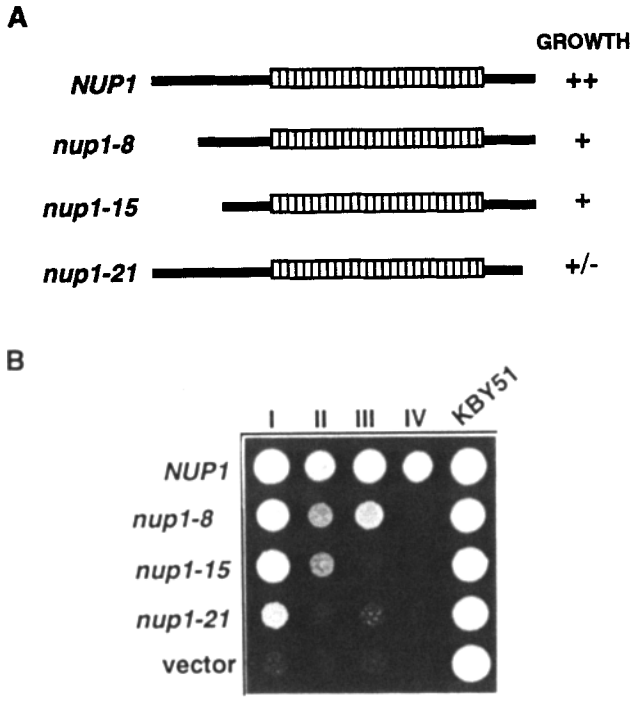

Figure 2. Allele specificity of *nle* mutants. (*A*) Diagram and growth rates of *NUP1* truncation mutants: *nupl-8*, Δa.a. 4–141; *nupl-15*, Δa.a. 4–191; *nupl-21*, Δa.a. 1,042–1,076 (Bogerd et al., manuscript in preparation). (*B*) Plasmids carrying *NUP1* (LDB107, *top*), the *nupl* truncation alleles (*middle*), or an empty plasmid (pRS314, *bottom*) were transformed into each *nle* mutant strain, as well as into the starting strain (*KBY51*, *last column*). Transformants were plated on media containing 5-FOA. Results are shown for a representative of each class. Class: *I*, *nle5*, *nle7*, *nlel0*; *II*, *nlel*, *nle8*, *nle9*, *nlel3*; *III*, *nle3*, *nle6*, *nlel7*; *IV*, *nle2*, *nle4*, *nlel1*, *nlel2*, *nlel4*, *nlel5*, *nlel6*.

We expected that an *snpl* mutation would belong to class I, because all of the *nupl* mutants tested are functional in an *snpl* strain. Therefore, we crossed each class I mutant to a *nupl::LEU2 snpl* strain (LDY176). Independent assortment of *nle* and *snpl* mutations would allow recovery of *NLE SNP1* spores (5FOA^r), whereas tight linkage between an *nle* mutant and *snpl* would result in failure to recover 5FOA^r spores. In one case (*nle5*), 0/50 segregants were found to be 5FOA^r, suggesting that *nle5* is tightly linked to the *SNP1* locus, and is likely to encode the suppressor. Characterization of this mutant is under way. Because the other class I mutants may cause synthetic lethality through indirect effects on *SNP1* function, they have not been further pursued.

nlel Is Allelic to srpl

nlel is a cold-sensitive mutant which showed allele-specific synthetic lethality with *nupl-21*. It also exhibited unlinked noncomplementation with several other mutants (see Table II). To isolate the *NLE1* gene, strain KBY44 was transformed with a library of yeast genomic DNA in a *TRP1* marked *CEN*-based vector (Connelly, M., and F. Hieter, unpublished) as described in Materials and Methods. 13 out of 10,000 colonies screened were capable of growth at 16°C, and sectoring was restored in all of these. Restriction mapping of plasmid DNA recovered from these colonies revealed three overlapping inserts (Fig. 3). A 2.5-kb HpaI-BglII subclone (pKBB11) was able to complement fully. To obtain sequence within the coding region, the right junction of the noncomplementing fragment in pKBB10 was sequenced. This sequence, as well as the restriction map, exactly matched that of *SRP1*, an essential yeast gene identified as a classical suppressor of an RNA polymerase I mutant (Yano et al., 1992). The complementing HpaI-BglII fragment spans the *SRP1* locus. To prove that *SRP1* is allelic to *nlel*, as opposed to a gene dosage suppressor, the *SRP1* locus was marked by integration of an *SRP1 HIS3* plasmid (pKBB13). This strain (KBY70) was then crossed to KBY44, and the meiotic products of 27 asci were examined. In all cases, cold sensitivity segregated with histidine auxotrophy, indicating linkage of the cold sensitive mutation to *SRP1*.

Srplp Is Physically Associated with Nuplp and Nup2p

The genetic interaction between *SRP1* and *NUP1* suggested that their respective gene products interacted physically and/or functionally within the cell. This is further supported by the observation of Yano et al. (1992) that the *SRP1* gene product is located at the nuclear envelope, and possibly within the NPC. To ascertain whether Nuplp and Srplp physically interact, we tested them using the "two-hybrid" system (Fields and Song, 1989). Fragments of *NUP1* were cloned into vector pGBT9, such that in-frame fusions were created between the DNA binding domain of the transcriptional activator Gal4p and either the amino-terminal ($Gal4_{DB}$-$Nupl_N$), repetitive ($Gal4_{DB}$-$Nupl_{rep}$), or carboxy-terminal ($Gal4_{DB}$-$Nupl_C$) domains of Nuplp. The entire *SRP1* coding region was cloned into pGAD424 to create a fusion with the

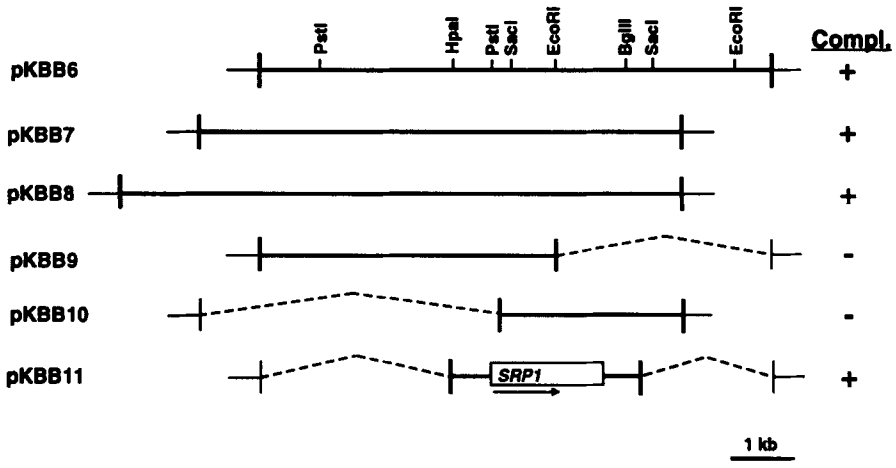

Figure 3. nle1 is allelic to *srp1*. Restriction maps of the three overlapping plasmids isolated by complementation of the cold sensitivity of *nle1* are shown at the top (pKBB6–8). pKBB9–11 contain the indicated subclones, and their ability to complement is shown at the right. Sequence obtained from the EcoR1 site of pKBB9 matched that of *SRP1*, as did the *nle1* restriction map. The *SRP1* open reading frame determined by Yano et al. (1992) is shown at the bottom.

Gal4p activation domain ($Gal4_{AD}$-Srp1). These constructs were co-transformed into yeast strain Y526, which contains the *lacZ* gene under control of the *GAL1* promoter. When plated on medium containing X-gal, cells transformed with both $Gal4_{DB}$-$Nup1_C$ and $Gal4_{AD}$-Srp1 turned blue, whereas neither construct was capable of activation when combined with an empty vector control (Fig. 4 *a*). Quantitation revealed an >160-fold increase in β-galactosidase activity (Fig. 4 *b*). Neither $Gal4_{DB}$-$Nup1_{rep}$ nor $Gal4_{DB}$-$Nup1_N$ showed activity in combination with $Gal4_{AD}$-Srp1 (data not shown).

We next sought biochemical evidence for the interaction between Nup1p and Srp1p. To this end, each of the three domains of *NUP1* was fused in frame to GST (Fig. 5 *a*). To detect interacting proteins, ^{35}S-labeled cell extracts from strain 1938 were incubated with each of the three GST-Nup1 fusions adsorbed to glutathione-Sepharose (Fig. 5 *b*). Several polypeptides were precipitated only by the amino-terminal fusion, and a protein of ~100 kD was specifically co-precipitated with the carboxy-terminal fusion. Three polypeptides (95, 67, and 55 kD) were co-precipitated by all three fusions, but not by GST alone. All of the fusion proteins include regions of the repeat domain (see Fig. 5 *a*),

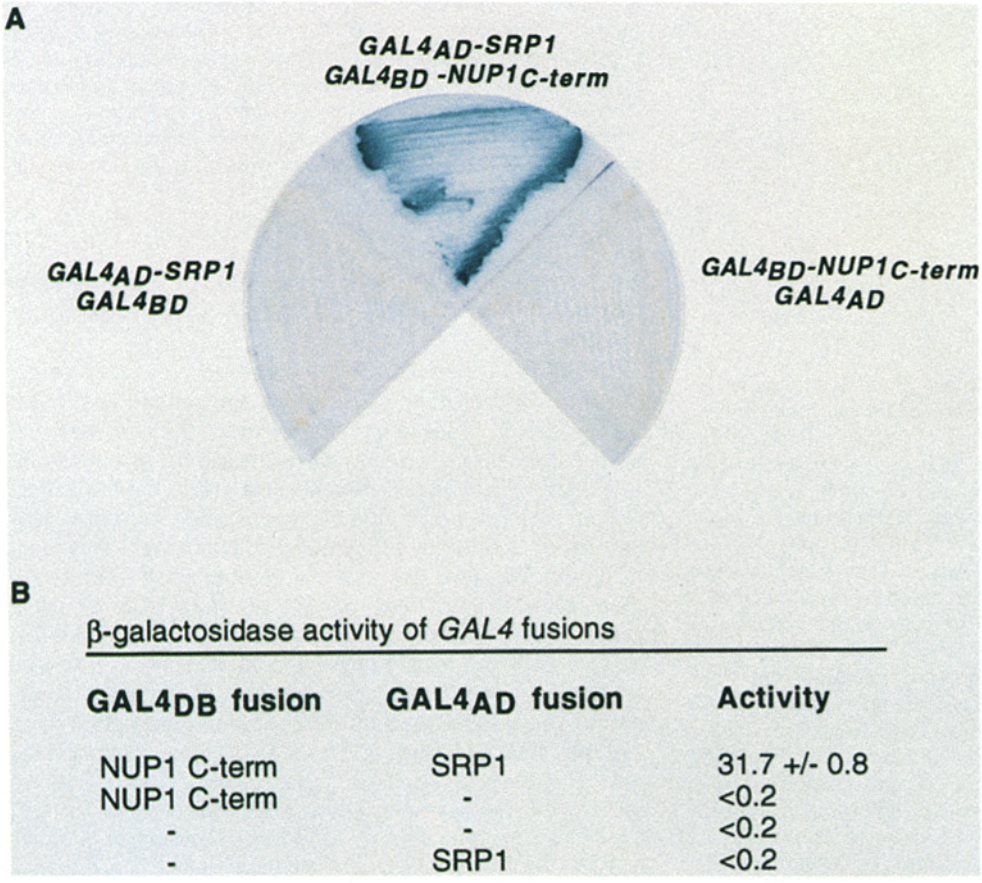

Figure 4. Interaction of Nup1p and Srp1p in the yeast two-hybrid system. Yeast strain Y526 was cotransformed with plasmids containing the indicated constructs. (*A*) Transformants were plated on media containing X-gal to indicate β-galactosidase production. (*Left*) $Gal4_{BD}$-Srp1 (pSWB17) and $Gal4_{BD}$ alone (pGBT9); (*middle*) $Gal4_{AD}$-Srp1 (pSWB-17) and $Gal4_{AD}$-$Nup1_{C\text{-}term}$ (pSWB11); (*right*) $Gal4_{BD}$-$Nup1_{C\text{-}term}$ (pSWB11) and $Gal4_{AD}$ alone (pGAD424). (*B*) β-galactosidase production was quantitated in each case by CPRG assay. Each assay was performed in triplicate.

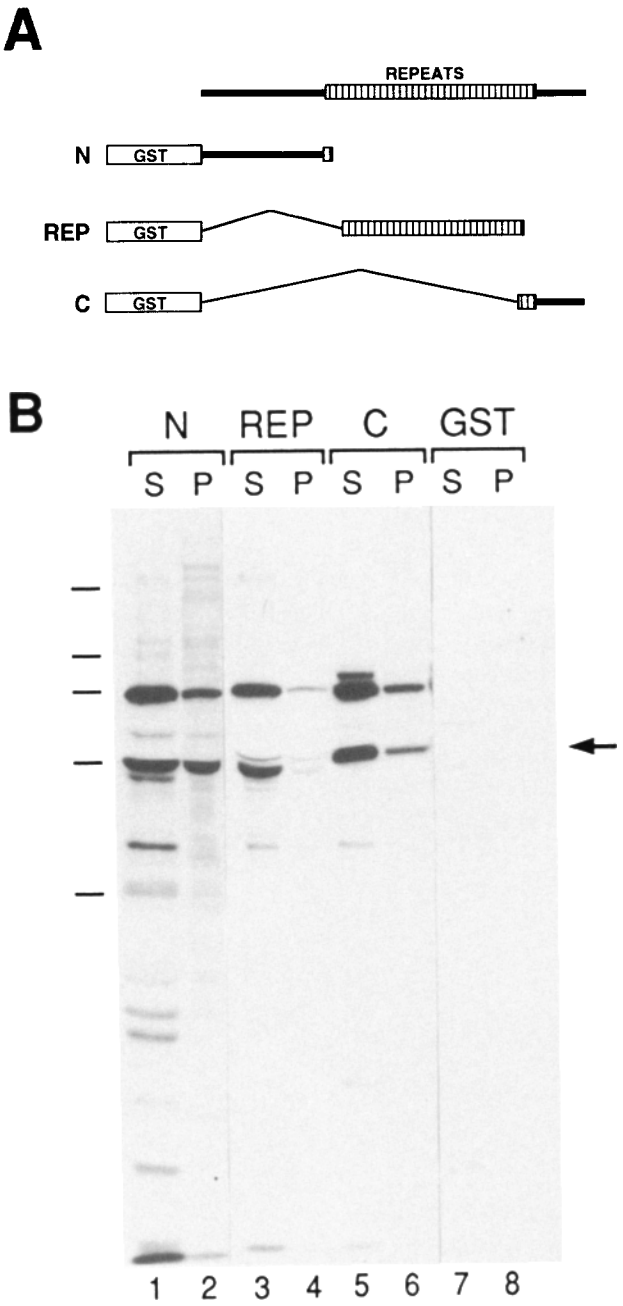

Figure 5. GST-Nupl precipitates a distinct set of yeast proteins. (*a*) Schematic of the GST fusion constructs used for precipitation from yeast extracts. Note that all of the fusions contain a region within the repetitive domain. The amino-terminal and repetitive domain constructs do not overlap, whereas the repetitive domain and carboxy-terminal domain constructs share 38 amino acids of repeat sequence. (*b*) ^{35}S-labeled yeast cells (strain 1938) were fractionated as described in Materials and Methods. The soluble fraction (*S*) represents proteins released into a low-speed supernatant after cell lysis. The particulate fraction (*P*) represents material released from the low speed pellet by extraction with buffer containing 1 M NaCl. All of the Nups are solubilized under these conditions. Fractions were incubated with GST fusions containing either the Nuplp amino-terminal domain (lanes *1* and *2*), central repetitive domain (lanes *3* and *4*), carboxy-terminal domain (lanes *5* and *6*), or GST alone (lanes *7* and *8*). Bound proteins were precipitated with glutathione-Sepharose, eluted with sample buffer, and analyzed by autoradiography after SDS-PAGE. The arrow at right refers to a band which migrates at the position expected of Srplp. Molecular weight standards denoted by arrows at left are as follows: skeletal muscle myosin (200 kd); β-galactosidase (116 kd); phosphorylase B (97.4 kd); BSA (66.2 kd); ovalbumin (45 kd).

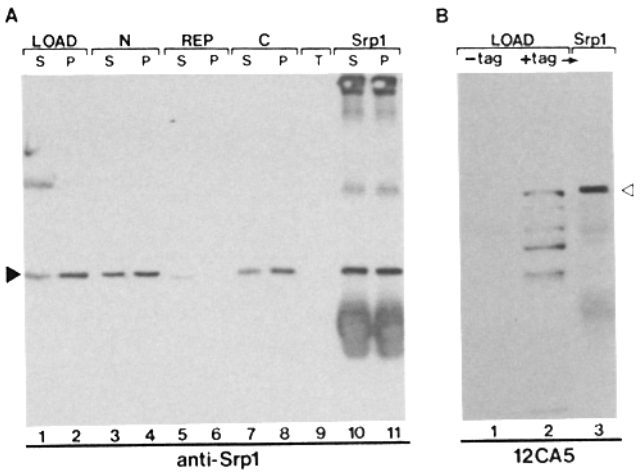

Figure 6. Co-precipitation of Nuplp and Srplp. (*a*) Unlabeled yeast cell fractions were prepared from strain 1938 as described in the legend to Fig. 5, and precipitated with glutathione-Sepharose-bound GST fusions containing either the amino-terminal (lanes *3* and *4*), central repetitive (lanes *5* and *6*), or carboxy-terminal (lanes *7* and *8*) domains of Nuplp, or GST alone (lane *9*, soluble and particulate fractions were combined in this case). Immunoprecipitation with anti-Srplp antiserum was performed in parallel (lanes *10* and *11*). Unprecipitated fractions were run in lanes *1* and *2*. Precipitated proteins were eluted in sample buffer, subjected to SDS-PAGE, and blotted to nitrocellulose. Blots were probed with anti-Srplp antiserum. Arrow at left points to the position of Srplp. Note that the relatively small amount of Srplp present in lanes *5* and *6* is probably artifactual. The GST-rep fusion protein, which is present in vast excess, migrates at roughly the same position (see gel artifact in Fig. 5), and may block efficient transfer of Srplp. (*b*) Unlabeled yeast strains were fractionated as described in the legend to Fig. 5. Total salt extracts from strain BJ926, which has untagged Nuplp (lane *1*) or LDY402, which carries an HA-tagged copy of Nuplp (lane *2*) were analyzed to show the position of the tagged Nuplp protein (*arrow, right*). The salt extract from strain LDY402 was immunoprecipitated with anti-Srplp antibody in parallel (lane *3*). After SDS-PAGE, proteins were transferred to nitrocellulose, and the blots were probed with mAb 12CA5, directed against the HA tag.

thus it is likely that the latter group of co-precipitating proteins interact through the repeats, either directly or indirectly.

The 67-kD band matched the predicted molecular weight of Srplp. To determine whether this polypeptide was in fact Srplp, Western blots of unlabeled extracts precipitated with each Nuplp fusion protein, or with anti-Srplp antiserum, were probed with anti-Srplp antiserum (Fig. 6 *a*). All three fusion proteins precipitated a polypeptide recognized by anti-Srplp, and having the same molecular weight as authentic Srplp. We were also able to co-immunoprecipitate epitope tagged Nuplp from extracts of strain LDY402 using anti-Srplp antibody (Fig. 6 *b*). These results suggest that Srplp and Nuplp form a physical complex, which may also contain a number of other polypeptides. We note that a portion of Srplp is released into the supernatant fraction upon cell lysis (Fig. 6 *a*, lane *1*). This is also true of Nsplp and the

mammalian nucleoporin p62. In the case of p62, all of the soluble protein can be chased into the particulate fraction, suggesting that the protein is made in excess and only slowly incorporated into the NPC (Davis and Blobel, 1986). This may also be true of Srplp and Nsplp. Alternatively, these proteins may simply be less tightly associated than other nucleoporins.

The allele-specific synthetic lethality of *srp1* with *nup1-21* suggested that the interaction might occur through the carboxy-terminal domain of Nuplp. The observation that only the carboxy-terminal $Gal4_{BD}$ fusion was capable of activating β-galactosidase in combination with $Gal4_{AD}$-Srpl supports this interpretation. However, all of the GST-Nupl fusions were able to precipitate Srplp from yeast extracts, conditions presumably requiring much higher affinity than that required for two-hybrid activation. Therefore, we think it likely that the lack of activity of amino-terminal and repeat domain fusions in the two-hybrid system results either from failure of these fusion proteins to enter the nucleus or from conformational constraints that prevent activation. Interestingly, the GST-rep fusion was least efficient at precipitating Srplp, particularly from the pellet fraction. Therefore, the repetitive domain alone appears to have a low affinity for Srplp, and cannot effectively compete with the endogenous Nuplp that is present in the pellet. Thus, although the repeats probably provide the binding site for Srplp (see below), the terminal domains may stabilize the interaction. The specificity of the genetic interaction may reflect this, or may simply be the result of synergistic effects resulting from compromising the function of two members of a complex.

Association of Srplp with Other Nucleoporins

Because Nuplp is a member of a family of related NPC proteins, we next asked whether other members of this family also associate with Srplp. To this end, fractionated cell extracts from yeast strain BJ926 were immunoprecipitated with anti-Srplp, and the bound proteins subjected to Western blot analysis with antibodies that recognize the nucleoporins (Fig. 7). None of the proteins recognized by mAb 192, including Nup49, 100, 116, and several uncharacterized Nups (Wente et al., 1992), were co-immunoprecipitated with anti-Srplp antibody (Fig. 7 *a*). However, mAb 414 (specific for Nsplp and Nup2p) recognized a co-immunoprecipitating protein migrating at the position of Nup2p (Fig. 7 *b*). To confirm the identity of this polypeptide, the experiment was repeated with strain LDY402, which carries a *NUP2* deletion. The co-precipitating polypeptide was no longer present. We conclude that Srplp is also associated in vivo with Nup2p.

Srplp, Nuplp, and Nup2p could associate as a single complex. Alternatively, Srplp could be a common component of two distinct complexes, each containing one of the nucleoporins. To distinguish between these two possibilities, Western blots of proteins precipitated by each of the GST-Nupl fusions were probed with mAb 414 (Fig. 8, lanes *8–13*). Nup2p was not co-precipitated by any of the fusion proteins, although all were capable of precipitating Srplp in a parallel experiment (see Fig. 5). Moreover, immunoprecipitation of full length Nupl from strain LDY74 failed to co-precipitate Nup2p (Fig. 8, lanes *2–5*). We conclude that Srplp is capable of binding either Nuplp or Nup2p, but not both simultaneously.

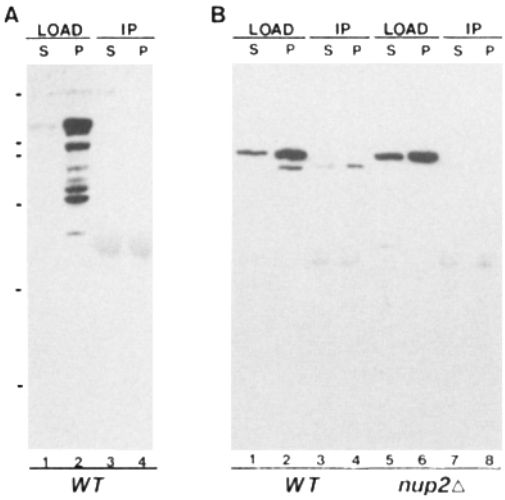

Figure 7. Srplp interaction with other nucleoporins. (*A*) Unlabeled yeast strain BJ926 was fractionated as described in the legend to Fig. 5. Total protein from each fraction was loaded in lanes *1* and *2*. In parallel, proteins from each fraction were immunoprecipitated with anti-Srplp antiserum. Bound proteins were eluted in sample buffer and loaded in lanes *3* and *4*. Proteins were transferred to nitrocellulose, and blots were probed with mAb 192, which recognizes several Nups, as can be seen in lane *2*. None of these are present in anti-Srplp precipitates. (*B*) Lanes *1–4* are identical to lanes *1–4* of *A*, except that blots were probed with antibody 414, which recognizes Nsplp and Nup2p. In this case, the lower band was also present in the anti-Srplp immunoprecipitate (lanes *3* and *4*). The position of this band corresponds to Nup2p, however variations in strains used and gel parameters can affect the relative migration of these two proteins. Therefore a strain deleted for Nup2p (LDY402) was used in lanes *5–8*. As expected, only the upper band (Nsplp) was present (lanes *5* and *6*), and the immunoprecipitable band was no longer apparent (lanes *7* and *8*).

Discussion

We have described the results of a genetic screen designed to identify proteins that are functionally related to the nucleoporin *NUP1*. We have characterized the first of these mutants, and found that it encodes a protein that physically associates with two of the nucleoporins, Nuplp and Nup2p, within the NPC.

We previously reported that *NUP1* is an essential gene (Davis and Fink, 1990). However, its requirement can be bypassed in some strains. Strain L3852 contains a bypass suppressor (*SNP1*) that segregates as a single gene. We do not know the frequency of this allele, or whether other loci can also cause suppression. Because Nuplp is a member of a family of proteins that may have partially redundant functions within the NPC, strains carrying variant alleles of some of these genes may exhibit differing requirements for any given one. For example, a deletion of *NUP2* has no phenotype and could therefore be lost through mutation, but the resulting strain would have a more stringent requirement for *NUP1* and *NSP1*, because normally viable mutations in these genes are synthetically lethal with Δ*NUP2* (Loeb et al., 1993). Such interactions appear to be fairly common among related gene families. We have not detected obvious differences in the constitution of the nucleoporins in *SNP1* as compared to *snp1* strains, as assayed by Western blotting with

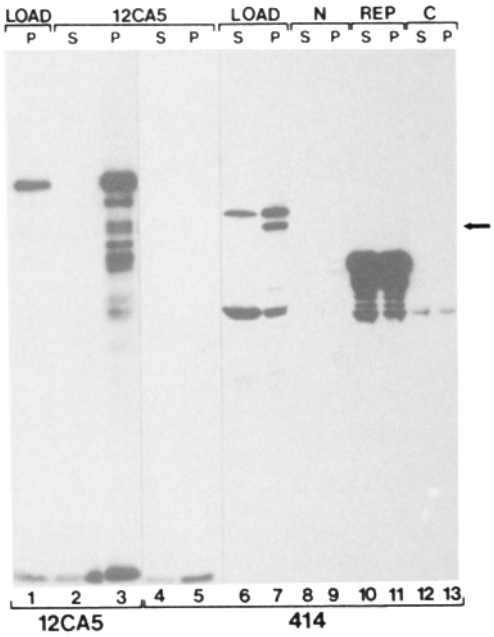

Figure 8. Nuplp and Nup2p do not co-immunoprecipitate. Yeast strain LDY74, which harbors an epitope tagged copy of Nuplp under GAL control (pLDB17) was induced for three hours on galactose and then fractionated as described in the legend to Fig. 5. Soluble (lanes *2* and *4*) and particulate (lanes *3* and *5*) fractions were immunoprecipitated with mAb 12CA5, and the bound proteins were eluted in sample buffer. Total protein from the particulate fraction was loaded in parallel (lane *1*). Proteins were transferred to nitrocellulose after electrophoresis, and the blots were probed with either mAb 12CA5 (lanes *1–3*) or mAb 414 (lanes *4* and *5*). Although mAb 12CA5 immunoprecipitated tagged Nuplp very efficiently, Nup2p was not coprecipitated, as no mAb 414-reactive proteins were present. Fusion protein precipitation gave the same result. Cell fractions from strain BJ926 were precipitated with each of the three GST-Nuplp fusion proteins, exactly as described in the legend to Fig. 6 (lanes *8–13*). Total protein was loaded in lanes *6* and *7*. Proteins were blotted to nitrocellulose after SDS-PAGE, and the blots were probed with mAb 414. Nup2p (*arrow*) was not precipitated by any of the Nuplp fusions, although all of them precipitated Srplp (see Fig. 6 *A*). The strong bands in lanes *10* and *11* represent binding of mAb 414 to the repetitive domain of Nuplp, which is present in very large quantity.

anti-nucleoporin antibodies. However more subtle changes cannot be ruled out.

Several of the mutants isolated in our screen exhibit unlinked (or nonallelic) noncomplementation, as evidenced by inability of the double heterozygote to grow in the absence of the wild-type *NUP1*. This phenomenon has been observed in a number of instances and may be explained several ways. As proposed for noncomplementing mutations in α and β tubulins, the doubly mutant heterodimer could exert a dominant effect by poisoning the tubulin polymer. Alternatively, the expected reduction in functional dimers from 50 to 25% in the double mutant could itself be lethal (Stearns and Botstein, 1988). In theory, mutations affecting different steps in linear or parallel pathways could also fail to complement, if the productivity of the pathway(s) falls below the threshold required for viability. Mutations that lower gene expression or activity nonspecifically could also cause many heterozygous mutants to become haplo-insufficient, if expression of the wild-type gene was just over the required threshold. The latter phenomenon most likely explains the failure of RNA polymerase II mutations to complement a *ubx* mutation in *Drosophila* (Mortin and Lefevre, 1981). While the explanation for noncomplementation among the *nle* mutants remains to be determined, we note that the NPC is a large hetero-oligomeric structure that is almost certain to have strict requirements for stoichiometry amongst its constituents. Thus, it is reasonable to suppose that at least some of the genes belonging to this class of mutants encode proteins that are functionally and/or physically associated. This model is favored by our demonstration that one of the mutants from this class maps to the *SRP1* locus, and that Srplp is physically associated with two of the nucleoporins.

We have shown that Srplp binds to both Nuplp and Nup2p. Our experiments suggest that Srplp forms distinct complexes with each, because the Nuplp co-precipitates Srplp but not Nup2p. We presume that Srplp binds to both proteins through the central repetitive domain, because this is the only sequence motif in common between the three domains of Nuplp (all of which are capable of binding Srplp) and between Nuplp and Nup2p. It is interesting that Nsplp which has a similar domain, does not appear to associate with Srplp. This may reflect a functional distinction between the highly conserved repeats of Nsplp and the more divergent ones found in Nuplp and Nup2p. It is also conceivable that binding to Srplp in one or more cases is indirect, occurring through other proteins that bind each of the domains specifically. The experiments that we have described do not rule out this possibility, and in fact suggest that several other proteins may be present in the Srplp–Nuplp complex.

Truncations of *NUP1* are inviable in combination with a *NUP2* deletion (Loeb et al., 1993). Our results argue against a model in which the presence of Nup2p is required strictly to stabilize Nuplp in a complex, because the two proteins do not appear to associate. A more likely alternative is that the two complexes have some functional redundancy. Another possibility is that the physical interactions between these proteins are dynamic, such that Srplp exchange between Nuplp and Nup2p complexes is important for function. We are currently investigating this model by examining Srplp-Nup complexes in various *nup* mutant backgrounds.

Mutations in *SRP1* have been isolated in a number of genetic screens. Some alleles are suppressors of mutations in RNA polymerase I which are thought to affect subunit assembly. Depletion of Srplp transforms the nucleolus, which normally forms a crescent immediately under the nuclear envelope, into a number of small foci in the nucleus (Nomura, M., personal communication). *SRP1* was also identified recently as a high copy suppressor of the *cse1* mutant, which is defective for mitotic chromosome segregation (Fitzgerald-Hayes, M., personal communication). These pleiotropic phenotypes could be explained by a primary defect in nuclear import of proteins required for each process. However, we have tested the *srp1* allele described here for import of an H2B-LacZ fusion protein and found no defect (Belanger and Davis, unpublished data), arguing against the idea that the primary defect involves nuclear import.

The data could also be simply explained if Srplp interaction with the nucleoporins provides a structural connection between the NPC and the underlying framework of the nu-

cleus, loss of which could disrupt the association of the nucleolus (and perhaps of mitotic chromosomes) with the nuclear envelope. The mammalian analogs of the Nups are predominantly found in the distal ring of the nucleoplasmic "fishtrap" which, at least in some organisms, is connected to a hexagonal filamentous lattice of unknown composition (Goldberg and Allen, 1992). The NPC is also thought to be connected to the nuclear lamina. Two observations lend support to the idea that Srplp-Nup interactions may be involved in connecting the NPC to the nuclear skeleton. First, the morphology of the nuclear envelope in *nup1* mutants suggests that it has detached from an underlying scaffold (Bogerd, A. M., J. A. Hoffman, D. C. Amberg, G. R. Fink, and L. I. Davis, manuscript submitted for publication). While NPC structure is not notably different from wild-type, the nuclear envelope forms long finger-like projections that extend out from the bulk of the nucleus, and often wrap around other cellular organelles. Second, Srplp has similarity to the β-catenin/armadillo/plakoglobin family (M. Nomura, personal communication). These proteins interact with the cytoplasmic domain of cadherins, and are thought to modulate cadherin aggregation and linkage to cytoskeletal elements at intercellular junctions (reviewed in Kemler, 1993). In an analogous fashion, Srplp could be the link between the NPC and an underlying nucleoskeletal component. We note that topology of the NPC is equivalent to that of junctional plaques; both are peripherally associated with the cytoplasmic/nucleoplasmic membrane face (as opposed to the lumenal/external face) through interactions with integral membrane proteins.

These observations suggest that the NPC may function as a structural component of the cytoskeleton, as well as a conduit for nucleocytoplasmic transport. Whether these functions are distinct or interdependent remains to be determined. Further insight into the interactions between NPC components and cytoskeletal elements should be gained by an examination of the proteins that interact with Srplp. Such studies will also help establish whether there is an evolutionary or functional link between the NPC and intercellular junctions.

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