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Yeast Nle3p/Nup170p Is Required for Normal Stoichiometry of FG Nucleoporins within the Nuclear Pore Complex

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The FG nucleoporins are a conserved family of proteins, some of which bind to the nuclear localization sequence receptor, karyopherin. Distinct members of this family are found in each region of the nuclear pore complex (NPC), spanning from the cytoplasmically disposed filaments to the distal end of the nuclear basket. Movement of karyopherin from one FG nucleoporin to the next may be required for translocation of substrates across the NPC. So far, nothing is known about how the FG nucleoporins are localized within the NPC. To identify proteins that interact functionally with one member of this family, the *Saccharomyces cerevisiae* protein Nup1p, we previously identified 16 complementation groups containing mutants that are lethal in the absence of *NUP1*. These mutants were referred to as *nle* (Nup-lethal) mutants. Mutants in the *nle3*/*nle17* complementation group are lethal in combination with amino-terminal *nup1* truncation mutants, which we have previously shown to be defective for localization to the NPC. Here we show that *NLE3* (which is allelic to *NUP170*) encodes a protein with similarity to the mammalian nucleoporin Nup155. We show that Nle3p coprecipitates with glutathione *S*-transferase fusions containing the amino-terminal domain of Nup1p. Furthermore, a deletion of Nle3p leads to changes in the stoichiometry of several of the XFXFG nucleoporins, including the loss of Nup1p and Nup2p. These results suggest that Nle3p plays a role in localizing specific FG nucleoporins within the NPC. The broad spectrum of synthetic phenotypes observed with the *nle3*Δ mutant provides support for this model. We also identify a redundant yeast homolog that can partially substitute for Nle3p and show that together these proteins are required for viability.

The nuclear envelope allows selective passage of nucleic acid and proteins between the nucleus and the cytoplasm via nuclear pore complexes (NPCs). The NPC is estimated to contain approximately 100 different polypeptides (nucleoporins), many of which have yet to be characterized. Some of the nucleoporins play a role in protein and/or RNA transport, whereas others probably function in the assembly and maintenance of the NPC structure (for reviews, see references 15, 36, and 46). In recent years tremendous progress has been made towards elucidating the mechanism by which macromolecular transport occurs. However, we still know relatively little about how the NPC is assembled and how specific nucleoporins are localized within the NPC.

Among the known NPC components is a conserved family of polypeptides termed the FG nucleoporins (for reviews, see references 15 and 53). The signature of this family is a domain consisting of short repeats centered around phenylalanine-glycine residues. *Saccharomyces cerevisiae* genes *NUP1*, *NUP2*, and *NSP1* and vertebrate genes *Nup62*, *Nup153*, *Nup214*, *Nup358*, and *Pom121* encode nucleoporins containing XFXFG repeats, whereas yeast *NUP49*, *NUP100*, *NUP116*, and *NUP145* and vertebrate *Nup98* encode proteins that share repetitive GLFG motifs. Still other family members, such as yeast Rip1p and Nup159p and mammalian Nup214 (CAN) contain more degenerate FG motifs. In metazoan cells, different members of the FG nucleoporin family are found in different regions of the NPC. Antibodies to Nup214 and Nup358 decorate filaments that extend from the NPC into the cytoplasm (31, 44, 65, 67). These filaments are thought to provide the initial docking site for proteins destined for nuclear import (51). At least one FG nucleoporin (Nup62) is localized in the central region of the NPC, apparently within the spoke-ring complex that makes up its core (44). Finally, Nup153 is localized on the distal annulus of the nuclear basket, which extends some 100 to 150 nm into the nuclear interior (14, 44). Analogous to the cytoplasmic filaments, the nuclear basket may be the site at which ribonucleoprotein particles dock prior to export (35). The disposition of the yeast nucleoporins within the NPC is not known. All of the FG nucleoporins except Pom121 (which is an integral membrane protein) can be extracted with a high concentration of salt or with urea (16, 52, 58), suggesting that they do not make up the core structural elements of the NPC.

Antibodies that recognize the FG nucleoporins inhibit both protein import and RNA export (21, 67). Moreover, mutation or deletion of many of the yeast FG nucleoporins leads to temperature-sensitive inhibition of either protein import or RNA export or both (10, 23, 33, 40, 41, 45, 55). More recently, several of these proteins have been shown to bind to either the α or the β subunit of the soluble nuclear localization sequence receptor complex called karyopherin, most likely through the FG repeat domain (5a, 6, 29, 48, 49). In vivo studies further suggest that the FG nucleoporins form sequential rather than redundant karyopherin-binding sites (32). Thus, binding and release of karyopherin to nucleoporins arrayed across the NPC could provide the mechanism by which translocation is effected. Several FG repeat proteins, including nucleoporins Nup2p and Nup358, contain one or more binding sites for the GTP-bound form of the small GTPase Ran (TC4) (5, 18, 65, 67), which is required for the translocation step of protein import (38, 39). Because antibodies to Nup358 and to Ran itself decorate the cytoplasmic NPC filaments (65, 67), it is likely that the Ran-binding nucleoporins provide the initial docking step for karyopherin. Recent evidence suggests that

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TABLE 1. Yeast strains used in this study

Strain	Genotype or relevant characteristic	Reference or source
MKY34[a]	*MAT***a** *nle3-1 ade2-101 ade3 his3Δ200 lys2-801 ura3-52 nup1-Δ2::LEU2* [pLDB73]	6
LDY433[b]	*MAT***a** *ade2-1 ura3-1 his3-11 trp1-1 leu2-3 can1-100*	R. Rothstein
LDY434[b]	*MATα ade2-1 ura3-1 his3-11 trp1-1 leu2-3 can1-100*	R. Rothstein
LDY436	**a**/α diploid from a cross between LDY433 and LDY434	This study
JRY9	*MATα nle3-Δ1::HIS3 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	This study
JPY21	*MATα nle3-Δ2::HIS3 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	This study
JPY23	*MAT***a** *nle3-Δ2::HIS3 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	This study
JPY44	*MAT***a** *yer105-Δ1::URA3 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	This study
JPY45	*MATα yer105-Δ1::URA3 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	This study
SWY3	*MATα nup100::URA3 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	62
LDY626	*MAT***a** *nup2-Δ1::TRP1 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	This study
LDY461	*MAT***a** *nup1-Δ2::LEU2 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	This study
LDY463	*MAT***a** *nle4-1 ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100*	27a
LDY618	*MAT***a** *srp1::KAN ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100* [*srp1-49 CEN TRP*]	This study
LDY619	*MAT***a** *srp1::KAN ade2-1 ura3-1 trp1-1 leu2-3 his3-11 can1-100* [*srp1-31 CEN TRP*]	This study
KBY223	*MATα srp1::KAN ade2-1 ura3-1 trp1-1 srp1-175::LEU2 his3-11 can1-100*	5a
KBY224	*MATα srp1::KAN ade2-1 ura3-1 trp1-1 srp1-183::LEU2 his3-11 can1-100*	5a
JPY41	c-*myc*-tagged *NLE3* integrated at *LEU2* in JRY9	This study

[a] Not isogenic with W303.
[b] LDY433 and 434 are isogenic W303 strains.

the Ran GTPase-activating protein (RanGAP) is required to release karyopherin from the docking site (37). Thus, Ran may control translocation by modulating binding and release between karyopherin and the FG repeats of the nucleoporins.

If correct, this model implies that the FG repeats may be generic binding sites for karyopherin, whereas the unique domains of each FG nucleoporin regulate karyopherin binding and release during translocation. It follows that specific localization of the FG nucleoporins within the NPC is crucial for efficient translocation. Presumably, the exact localization of each nucleoporin within the NPC is also defined by a unique domain. Although truncation analysis of a number of yeast FG nucleoporins suggests that this is the case (10, 20, 42), nothing is known regarding the way in which these proteins are tethered to specific regions of the NPC. Candidate "anchors" for the FG nucleoporins include a number of NPC proteins that are unrelated to the FG repeat family, including peripheral proteins Nic96p (24) and Nup82p of *S. cerevisiae* (25, 27a, 28), rat Nup155 (47), and rat Nup180 (Tpr) (13) and integral membrane proteins Pom152p of *S. cerevisiae* (64) and rat Pom210 (63). Nic96p, Nup82p, and Nup180 all have regions predicted to form coiled coils and thus could form a structural link to the FG nucleoporins, many of which themselves have coiled coil domains. Furthermore, Nic96p and Nup82p can be coimmunoprecipitated with the XFXFG nucleoporin Nsp1p (24–26) and *NUP82* is allelic with *nle4*, a mutation that is lethal in the absence of the XFXFG nucleoporin Nup1p (27a). While these results suggest that some of the novel nucleoporins function together with the FG repeat family, the exact role that each of these proteins plays in NPC function has not been elucidated.

In order to identify proteins that interact functionally with the FG nucleoporins, we previously identified *S. cerevisiae* mutants (*nle* [Nup-lethal] mutants) that fall into 16 complementation groups and that are inviable in combination with a deletion of the yeast XFXFG nucleoporin *NUP1*. *NLE1* is allelic with *SRP1* (6) and encodes the yeast homolog of karyopherin α. *NLE7* is allelic with *YRB1* (32), which encodes a small Ran-GTP-binding protein that stimulates the activity of the RanGAP, Rna1p (7, 32, 50). Finally, *NLE4* is allelic to *NUP82* (27a). Here we show that *NLE3* encodes a homolog of the novel mammalian nucleoporin Nup155. *NLE3* is allelic to a yeast gene recently identified as *NUP170* (1). The combined function of Nle3p and a redundant yeast protein encoded by the *YER105* (*NUP157*) locus is essential for viability. We also show that Nle3p associates specifically with the region of Nup1p required for its assembly into the NPC and that the stoichiometries of several FG nucleoporins are altered when *NLE3* is deleted.

MATERIALS AND METHODS

Reagents. Enzymes for molecular biology were purchased from Boehringer Mannheim (Indianapolis, Ind.), Pharmacia (Piscataway, N.J.), and New England Biolabs (Beverly, Mass.). Lyticase was purchased from Enzogenetics (Corvallis, Oreg.). 5-Fluoro-orotic acid (5FOA) was purchased through the Genetics Society of America consortium. Antibody 9E10 ascites fluid was obtained from the Duke Cancer Center hybridoma facility. The yeast genomic library carried in pRS200 was furnished by Phil Hieter. Phage stock containing the rat Nup155 gene, as well as anti-Nup155 antisera, was provided by A. Radu and G. Blobel.

Strains, media, and standard techniques. *S. cerevisiae* strains used are listed in Table 1. DNA cloning was performed by standard techniques outlined by Sambrook et al. (54). Yeast cell culture and genetic manipulations were performed essentially as described by Sherman et al. (57). All gene replacements were performed in an isogenic W303 background, and existing mutants were backcrossed several times into this background prior to performing crosses. Yeast shuttle plasmids and linear fragments were introduced into yeast cells by lithium acetate transformation (30). Selection against Ura^+ strains was accomplished by culture on solid synthetic medium containing 1 mg of 5FOA per ml (9). Sectoring assays were performed on solid synthetic medium containing one-fourth the normal amount of adenine and histidine supplements.

Nucleic acid methods. Standard procedures were used for restriction digestions and plasmid constructions. All DNA fragments were purified from agarose prior to cloning. DNA transformation into *Escherichia coli* DH5α was performed by standard procedures (54). Double-stranded plasmid DNA was sequenced with a Sequenase kit (United States Biochemical Corp.). The sequencing vector pBluescript (Stratagene) was used to subclone certain restriction fragments of *NLE3*, and the sequence was obtained with universal sequencing primers. Oligonucleotides were synthesized and used as primers to complete some regions. DNA data analysis was performed with software from DNASTAR (Madison, Wis.). Protein sequence analysis and alignments were performed with the BLAST algorithm (3) and the BestFit and PileUp programs (Genetics Computer Group, Madison, Wis.).

Cloning *NLE3* and *YER105*. The colony sectoring assay used to identify mutants synthetically lethal with *nup1* has been described previously (6). The *NLE3* gene was cloned by complementation of the Sec^- 5FOA-sensitive phenotype of strain MKY34 (*nle3-1 nup1::LEU2 ade2 ade3* [*NUP1 CEN URA3 ADE3*]) with a *TRP1 CEN ARS* yeast genomic library in pRS200. Transformants were plated on synthetic medium lacking Trp, and plates were incubated at room temperature for 1 week. Approximately 60,000 colonies were screened to identify those in which sectoring had been restored. Sectoring colonies were collected and further

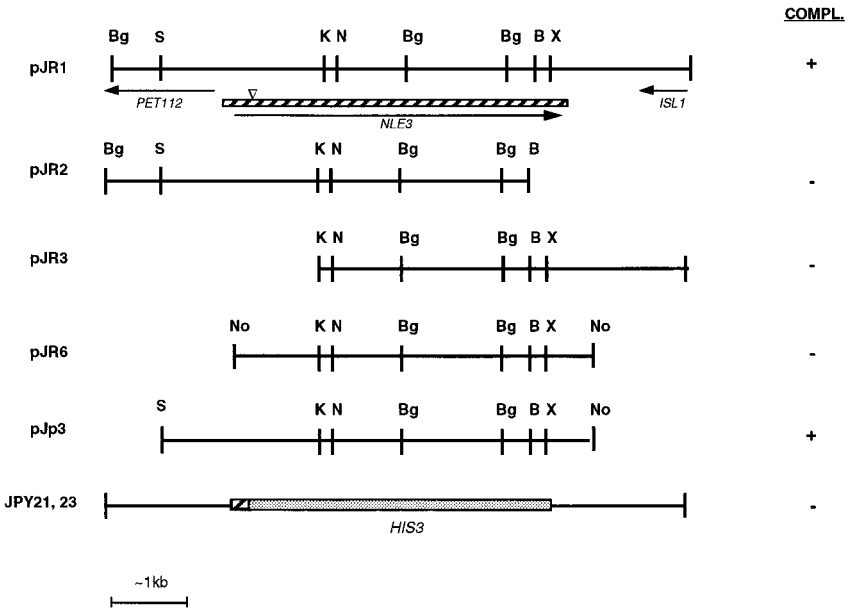

FIG. 1. Complementation of the *nle3-1* mutant. Overlapping inserts were subcloned from pJR1, which was isolated from a yeast genomic library by complementation of the 5FOA-sensitive Sec$^-$ phenotype of strain MKY34 (*nle3 nup1*::*LEU2 ade2 ade3 ura3* [*NUP1 CEN URA3 ADE3*]). The regions of *NLE3* (hatched boxes) that were replaced by the *HIS3* gene (not drawn to scale) are indicated. Arrows show the direction of each open reading frame. The approximate insertion site of the c-*myc* epitope is designated by the open arrowhead in pJR1. The ability or lack of ability of each construct to complement MKY34 is shown on the right as + or −. Restriction sites are as follows: B, *Bam*HI; Bg, *Bgl*II; K, *Kpn*I; N, *Nco*I; No, *Not*I; X, *Xba*I.

screened for 5FOA resistance. Complementing plasmids were then isolated and reintroduced into a fresh isolate of MKY34. Restriction analysis indicated that 30 of these contained the *NUP1* gene. The remaining two plasmids were identical and are referred to as pJR1 (Fig. 1). Initially, a plasmid containing a partial *NLE3* gene was constructed by thermal cycling with oligonucleotide primers containing *Not*I sites flanking the *NLE3* gene. The amplification product was then cloned into the *Not*I site of pRS305 to generate pJR6. We later determined that the open reading frame actually extended past the 5′ end of this clone. To remedy this, a fragment of pJR1 from an upstream *Spe*I site to the unique *Nco*I site in *NLE3* was subcloned into pJR6 to generate pJP3. An *Spe*I-*Not*I fragment containing full-length *NLE3* was then cloned from pJR3 into pRS316 (*CEN URA3*) to yield pJP13.

Oligonucleotide primers specific to sequences flanking *YER105* (GenBank accession number U18839) were used to amplify an 828-bp fragment (from nucleotide −236 to nucleotide +592) by thermal cycling. The fragment was hexamer labeled (22) with [^{32}P]dCTP and used to screen through ~15,000 *E. coli* colonies transformed with a yeast genomic library in pRS200 (*TRP1 CEN ARS*) (54). Ten overlapping plasmids were obtained, one of which (pJP8) contained the full-length *YER105* gene, as confirmed by restriction mapping and sequencing.

Gene disruptions. A partial disruption of the *NLE3* gene (*nle3-Δ1*) was made by replacing a 1.4-kb *Bgl*II-*Bgl*II fragment from pJR3 with the *HIS3* gene on a *Bam*HI fragment. A linear fragment of *nle3*::*HIS3* was then removed from the plasmid by digestion with the *Nco*I and *Xba*I enzymes and transformed into the W303 diploid strain LDY436. The disruption was confirmed by PCR with primers homologous to regions of *NLE3* outside the region used to transform. Sporulation and tetrad dissection yielded haploid strain JRY9. Replacement of the entire *NLE3* gene by *HIS3* (*nle3-Δ2*) was performed as described by Baudin et al. (4). Oligonucleotides, containing *HIS3* sequence from the 5′ and 3′ coding regions, flanked by 5′ and 3′ noncoding sequences from the *NLE3* gene, were designed. PCR amplification was then used to generate an *HIS3* gene fragment with ends homologous to *NLE3*. This fragment was purified and transformed directly into the **a**/α diploid strain LDY436, derived from isogenic W303 strains. PCR was used to confirm the correct integration of the *HIS3* gene and the replacement of the entire *NLE3* gene coding region (Fig. 1). Sporulation and tetrad dissection allowed recovery of isogenic *MAT***a** and *MAT*α *nle3*::*HIS3* spores (JPY23 and JPY21, respectively). *YER105* was disrupted in the same manner, except that the *URA3* gene was used instead. Tetrad dissection of this diploid yielded *yer105*::*URA3* spores JPY44 and JPY45.

Construction of *GAL*::*NUP155*. A 4.3-kb fragment from the *Rattus norvegicus* Nup155 gene was isolated by thermal cycling with phage containing the full-length gene as the template and primers designed to precisely amplify the Nup155 coding region. The resulting fragment was subcloned behind the yeast *GAL1,10* promoter in pMK27 (pRS314 with *GAL1,10* cloned into the polylinker) to generate pMK32. An antibody directed to a peptide region within Nup155 (47) was used to confirm galactose-specific expression in yeast cells. The pMK32 plasmid was tested for complementation of the cold-sensitive phenotype of the *nle3*Δ strain by transformation into strain JPY21 (*nle3*::*HIS3*). We observed no difference in growth rates between cells transformed with pMK32 versus plasmid alone when assayed on galactose at 18°C. The plasmid was also tested for complementation of the synthetic lethality between *nup1*Δ and *nle3*Δ by transforming it into an LDY460 × JPY23 diploid (*NUP1*/*nup1*::*LEU2 NLE3*/*nle3*::*HIS3*) and dissecting tetrads onto galactose-containing plates. No Leu$^+$ His$^+$ spores were obtained. In a similar manner, the rat Nup155 was assayed for complementation of the synthetic lethality between *nle3*Δ and *yer105*Δ with a cross between strains JPY23 and JPY45.

Epitope tagging of *NLE3*. *NLE3* was tagged by the insertion of the 10-amino-acid c-*myc* epitope (19) after amino acid 107 (Fig. 1) by a PCR strategy described by Vallette et al. (59). 3′ and 5′ primers homologous to the insertion site were designed, each containing a sequence encoding the c-*myc* tag. These were then used in separate amplification reactions, paired with a primer from the upstream polylinker in one case and a primer flanking the unique *Nco*I site in *NLE3* in the other. The two pieces of *NLE3* thus generated overlap at the ends containing the tag and were then used as a template to amplify the entire tagged construct with the two outside primers. The resulting fragment was digested with *Spe*I and *Nco*I and subcloned into pJP3 to generate pJP15. Proper placement of the tag was confirmed by sequence analysis. To determine whether the tagged construct was functional, pJP15 was transformed into yeast strain MKY34 and tested for restoration of the sectoring phenotype and the ability to grow on 5FOA.

Construction of GST fusion proteins. Construction of glutathione *S*-transferase (GST) fusions containing Nup1p amino acid residues 5 to 385 (amino terminal, pSWB1), 432 to 816 (repeats, pSWB5), and 778 to 1076 (carboxyl terminal, pSWB6) was described previously (6). To construct amino-terminal fusions with endpoints corresponding to the *nup1-8*, *nup1-15*, and *nup1-9* truncation mutants (see reference 10), oligonucleotides identical to those previously described (6) were used to amplify fragments from pB2291 (*nup1-8*), pLDB33 (*nup1-15*), and pLDB27 (*nup1-9*). These fragments were then cloned into the BamHI-EcoRI sites of pGEX-2TK to generate pSW2 (*GST*::*nup1*$^{142-385}$), pSW3 (*GST*::*nup1*$^{192-385}$), and pSW4 (*GST*::*nup1*$^{213-385}$), respectively.

Protein analysis. Yeast spheroplasts were subjected to hypotonic lysis and fractionation by low-speed centrifugation and extraction with 1 M NaCl exactly as described by Bogerd et al. (10). Proteins were separated electrophoretically on sodium dodecyl sulfate (SDS)–8% polyacrylamide gels and were transferred to nitrocellulose with a Bio-Rad semidry transfer apparatus. Immunoblotting procedures were identical to those previously described (6). Antibodies were used at the following dilutions: monoclonal antibody (MAb) 414 ascites fluid, 1:1,000; MAb 306 concentrated culture supernatant, 1:20; MAb 192 concentrated culture supernatant, 1:100; and MAb 9E10 ascites fluid, 1:500.

Precipitation of 1 M salt extracts containing *myc*-tagged *NLE3* was performed essentially as previously described (6). Briefly, 1 M salt extracts (S2 fractions)

were made as described above, diluted to a final buffer composition of 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.4)–140 mM NaCl–5 mM EDTA–1 mM dithiothreitol–0.1% Triton X-100–1% milk, and cleared by centrifugation at 100,000 × *g* for 15 min at 4°C. An amount of supernatant derived from starting material with a total optical density at 600 nm (OD_{600}) of 3.5 was then used for precipitation with each GST fusion protein. To prepare beads for precipitation, equal amounts of each GST fusion were adsorbed to 10 μl of glutathione beads in 50 mM HEPES (pH 7.4)–140 mM NaCl–5 mM EDTA–0.1% Triton X-100–1 mM dithiothreitol (wash buffer) plus 1% milk for 1 h at 4°C. The beads were washed three times with wash buffer prior to incubation with the diluted S2 fractions at 4°C for 2 h. After the last incubation, beads were washed five times with wash buffer and then eluted with SDS sample buffer. Samples were electrophoresed, blotted, and probed with MAb 9E10 as described above. Protease inhibitors (see reference 10) were included at all steps prior to elution in SDS buffer.

RESULTS

***nle3* shows allele-specific synthetic lethality with *nup1*.** The 16 *nle* complementation groups fell into four classes on the basis of their behavior when combined with different *nup1* truncation alleles (6). One class, which includes *nle3*, *nle6*, and *nle17*, was complemented only by the less severe amino-terminal truncation, *nup1-8*. *nle3* and *nle17* displayed tight linkage, and a DNA fragment isolated by complementation of the *nle3* mutant (see below) was also capable of rescuing *nle17*, suggesting that *nle3* and *nle17* are allelic. The *nle3*/*nle17* and *nle6* complementation groups were of interest because previous results suggested that the amino-terminal domain of Nup1p is required for its localization (10). Thus, the proteins encoded by these two genes could function in the assembly of Nup1p into the NPC.

Cloning and sequencing *NLE3*. To clone the *NLE3*/*NLE17* gene, we transformed a yeast genomic library into MKY34 (*nle3-1 ade2 ade3 trp1 nup1*::*LEU2* [*NUP1 CEN URA3 ADE3*]) and identified one plasmid (pJR1) containing an 8-kb insert capable of restoring sectoring and 5FOA resistance (see Materials and Methods). Sequences obtained from each end showed that the insert contained a carboxyl-terminal fragment of the *PET112* gene at one end and a small fragment of the *ILS1* gene at the other (Fig. 1). Subcloning and deletion analysis narrowed the complementing region to a 4.5-kb fragment between these two genes (Fig. 1). This region was sequenced and found to contain an open reading frame of ~4.0 kb. The sequence matches that which was subsequently deposited by the genome sequencing project (GenBank accession number X79489). A search of GenBank with the BLAST program (3) revealed that Nle3p has similarity to a previously identified mammalian nucleoporin termed Nup155 (47), as well as to an unidentified yeast open reading frame, *YER105*. Best Fit analysis showed that Nle3p is 48% similar and 23% identical to Nup155p and 64% similar and 44% identical to Yer105p. Yer105p has 45% similarity and 21% identity with Nup155p (Fig. 2).

***NLE3* and *YER 105* are functionally redundant.** To determine whether *NLE3* is essential for cell viability, we replaced one allele of *NLE3* with the *HIS3* gene in diploid strain LDY436 (Fig. 1). Sporulation and tetrad analysis of the diploid transformants allowed recovery of viable His^+ haploid spores (JPY21 and JPY23), showing that *NLE3* is not essential for viability. However, the His^+ spores displayed a leaky cold-sensitive phenotype at 18°C (Fig. 3). We performed a similar analysis with a deletion of *YER105* (JPY45). In this case, we observed no phenotype upon disruption (Fig. 3). Given the similarity between the two gene products and the fact that neither is essential, we next investigated whether these two gene products are functionally redundant by crossing isogenic strains JPY23 (*nle3-Δ1*::*HIS3*) and JPY45 (*yer105-Δ1*::*URA3*) and examining the phenotypes of double *nle3Δ yer105Δ* mutant segregants. Of 34 tetrads dissected, we recovered no viable Ura^+ His^+ spores (Table 2). Ura^+ His^+ spores were recovered when the JPY45-JPY23 diploid was first transformed with either pJP8 (*CEN TRP1 YER105*) or pJR1 (*CEN TRP1 NLE3*). In these cases, all of the double mutants were Trp^+. We conclude that Nle3p and Yer105p encode redundant proteins that together carry out an essential function.

To determine if rat Nup155 can substitute for either *NLE3* or *YER105*, we expressed rat Nup155 in yeast cells under the control of the inducible yeast *GAL1,10* promoter. Although a galactose-inducible protein of the predicted molecular weight was expressed, this protein did not rescue the synthetic lethality between *nup1Δ* and *nle3Δ* or that between *nle3Δ* and *yer105Δ*, whereas a *GAL*::*NLE3* construct expressed at similar levels was able to complement the synthetic lethality (data not shown; see Materials and Methods). Expression of mammalian Nup153p was also unable to complement the slow growth phenotype associated with the *nle3Δ* mutation.

Interactions with other known nucleoporins. To further explore the genetic relationships among *NLE3*, *YER105*, and other nucleoporins, the *nle3Δ* mutant (JPY21) was crossed with a series of isogenic mutant strains, including those with deletions of XFXFG nucleoporins Nup1p (LDY461) and Nup2p (WHY1) and of GLFG nucleoporin Nup100p (SWY3), as well as of a temperature-sensitive allele (*nle4-1*) of the novel nucleoporin Nup82p (LDY463). Sporulation and tetrad dissection of the resulting diploids revealed that *nle3Δ* was lethal in combination with *nup1Δ*, *nup2Δ*, and *nle4-1* but not with *nup100Δ* (Table 2). Deletion of *YER105* was also synthetically lethal with *nup1Δ* and *nle4-1* but had no synthetic phenotype in combination with *nup2Δ* (Table 2).

We also tested for genetic interactions between *nle3Δ* and *yer105Δ* and soluble components that mediate translocation. One of these is the α subunit of karyopherin (Srp1p), which is thought to translocate substrates across the NPC by binding to and releasing from sequentially arrayed FG nucleoporins. Several temperature-sensitive alleles of *SRP1* have been identified, all of which map to the central *arm* repeats of the protein (66). The tightest allele is *srp1-31*; *srp1-49* is somewhat leakier. An initial attempt to cross strains LDY619 (*srp1*::*KAN* [*srp1-31 CEN TRP1*]) and LDY618 (*srp1*::*KAN* [*srp1-49 CEN TRP1*]) to JPY21 (*nle3Δ*::*HIS3*) gave puzzling results, in that we were unable to recover either plasmid-borne *srp1* allele in combination with *nle3Δ*, even in spores that should contain the wild-type *SRP1* allele at the chromosomal locus (Table 2). To investigate the possibility that *srp1-31* and *srp1-49* are dominant in an *nle3Δ* background, we transformed strain JPY21 (*nle3Δ*::*HIS3*) with pJP13 (*NLE3 CEN URA3*) and then with plasmids pNOY163 (*srp1-31 CEN TRP1*) and pNOY166 (*srp1-49 CEN TRP1*) (66). We then assayed the phenotype of each *nle3Δ* *SRP1-srp1*^{ts} combination by selecting against the wild-type *NLE3* gene with 5FOA. Although fully recessive when a wild-type copy of *NLE3* is present, both *srp1-31* and *srp1-49* conferred dominant lethality in the absence of Nle3p (Fig. 4A). *srp1-49* was lethal at all temperatures tested, whereas *srp1-31* showed very slow growth at 23 and 30°C.

We also tested two cold-sensitive alleles of *SRP1* for dominant effects: the *nle1*/*srp1-183* allele, which has a point mutation in the amino-terminal domain (5a, 6), and the *srp1-175* allele, which has two point mutations near the carboxyl terminus (5a). To this end, we crossed JPY23 (*nle3*::*HIS3*) to KBY223 (*srp1*::*KAN srp1-175*::*LEU2*) and KBY224 (*srp1*::*KAN srp1-183*::*LEU2*). Tetrad dissection yielded no Kan^r His^+ Leu^+ spores in either case (Table 2), suggesting that these *srp1* alleles are also synthetically lethal with *nle3Δ*. However, neither of them is dominant, as evidenced by the recovery of

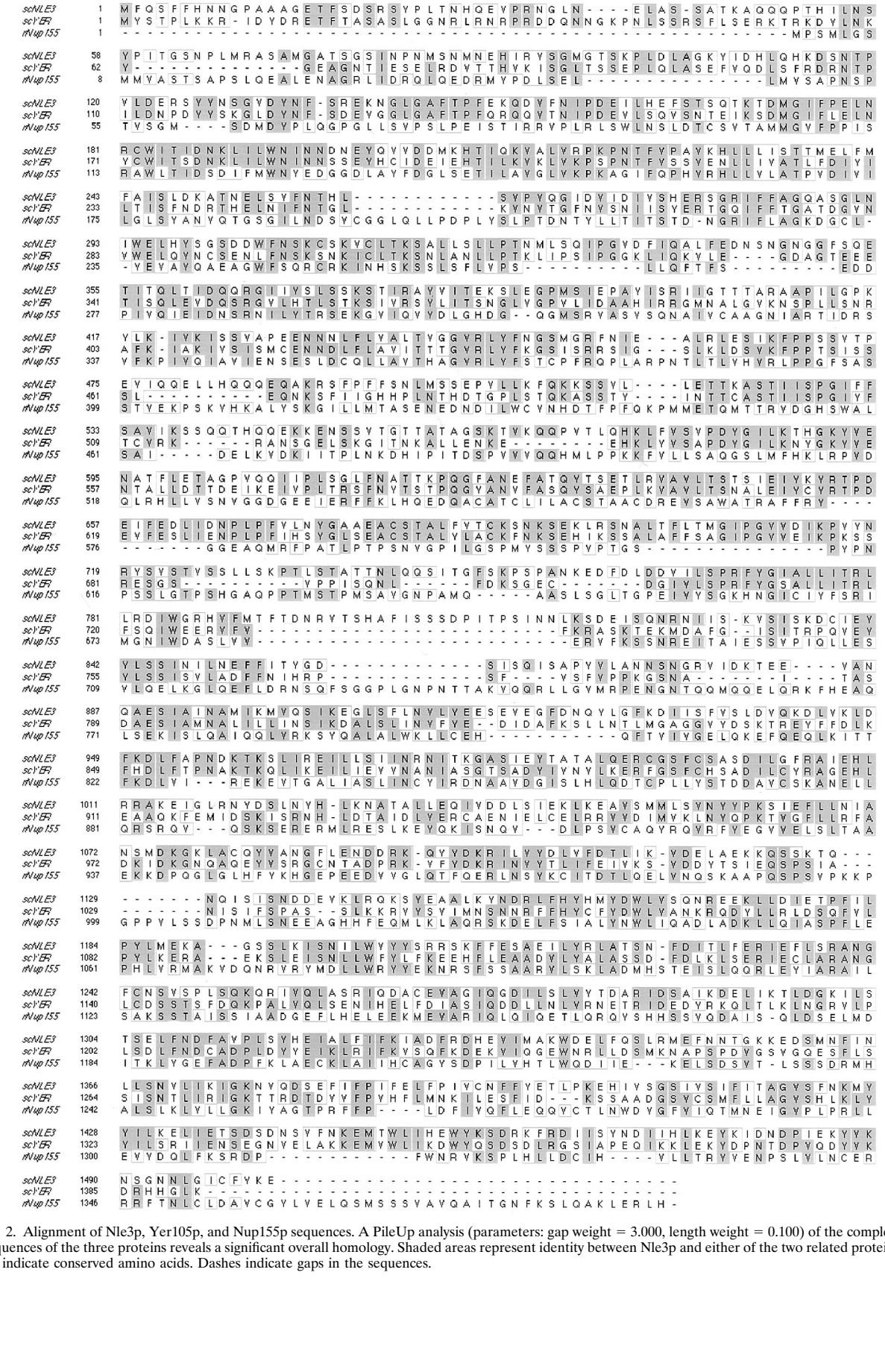

FIG. 2. Alignment of Nle3p, Yer105p, and Nup155p sequences. A PileUp analysis (parameters: gap weight = 3.000, length weight = 0.100) of the complete amino acid sequences of the three proteins reveals a significant overall homology. Shaded areas represent identity between Nle3p and either of the two related proteins; boxed regions indicate conserved amino acids. Dashes indicate gaps in the sequences.

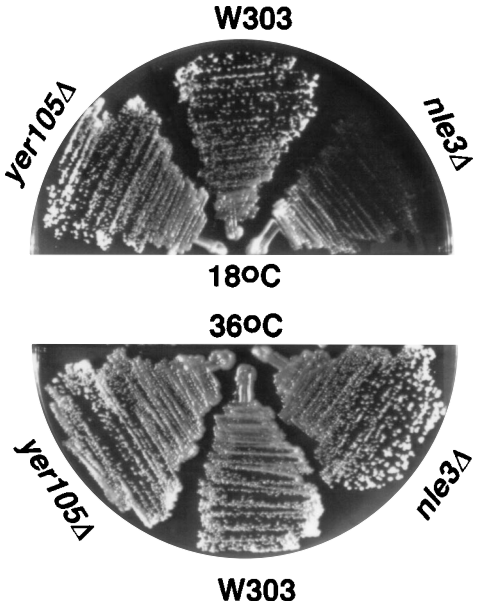

FIG. 3. Growth phenotypes of *NLE3* and *YER105* disruption mutants. Isogenic strains LDY433 (W303 wild type), JPY21 (*nle3-Δ1*::*HIS3*) and JPY45 (*yer105-Δ1*::*URA3*) were plated on rich medium (yeast extract-peptone-dextrose) and grown at either 18°C (top) or 36°C (bottom).

normal numbers of Kan^s His$^+$ Leu$^+$ spores. Thus, the dominant phenotype appears to be confined to mutations within the *arm* repeats of Srp1p. All of the *srp1* mutants were also tested for interactions with *yer105*Δ, by crossing them with strain JPY45 (*yer105*::*URA3*). All of these crosses produced viable double mutants at the expected frequencies (Table 2). Except for the *yer105*Δ *srp1-175* double mutant, whose phenotype was indistinguishable from that of the *srp1-175* single mutant, all of the double-mutant combinations grew somewhat more slowly than either mutant alone (data not shown). Thus, although there is some interaction between *yer105*Δ and *srp1* mutants, it is much less pronounced than that which we observed with *nle3*Δ.

The second effector we tested was Yrb1p, which is the yeast homolog of the small Ran-GTP-binding protein, RanBP1 (12, 43). Yrb1p increases the activity of the RanGAP, Rna1p (8, 56). Changes in Yrb1p and Rna1p functions have different effects in different nucleoporin deletion backgrounds; overexpression of *YRB1* suppresses *nup1*Δ and exacerbates *nup2*Δ growth defects, whereas both *rna1-1* and *yrb1* (*nle7-1*) mutants are lethal in the absence of Nup1p but have no effect in a *nup2*Δ strain (11, 32). These differences may reflect the functions of the two nucleoporins at distinct steps of docking and/or translocation. To determine whether the *nle3*Δ mutant is also sensitive to the level of *YRB1*, we transformed strain JPY21 (*nle3*Δ) with *YRB1* on a 2μm plasmid. Initial attempts to grow transformants at 30°C yielded no viable colonies. However, we were able to obtain slowly growing transformants upon incubation at 36°C. These transformants were inviable when assayed for growth at temperatures below 36°C (Fig. 4B). Thus, overexpression of *YRB1* greatly exacerbates the cold-sensitive phenotype of the *nle3*Δ mutant. We also examined the phenotype of the *nle3*Δ *rna1-1* double mutant by crossing LDY544 (*rna1-1*) with JPY23 (*nle3*Δ). Double mutant (Ts$^-$ His$^+$) spores were recovered at the expected frequency and had growth characteristics indistinguishable from those of the single *rna1-1* mutant (data not shown). These results suggest that the *nle3*Δ mutant is sensitive to increased but not decreased RanGAP activity, although it remains to be proven that Yrb1p functions solely to augument GAP activity in vivo.

Deletion of *NLE3* changes the stoichiometry of XFXFG nucleoporins in the NPC. The *nle3-1* mutant is one of two *nle* mutants that showed allele specificity with regard to amino-terminal truncations of Nup1p. Because we had previously shown that this domain of Nup1p is required for its localization to the NPC (10), we next investigated the possibility that Nle3p is involved in the assembly of FG nucleoporins within the NPC. To this end, we probed total extracts and crude subcellular fractions of *nle3*Δ, *yer105*Δ, and an isogenic wild-type strain with MAbs that recognize the XFXFG nucleoporins (MAbs 414 and 306 [17, 34]) or the GLFG nucleoporins (MAb 192 [62]). Consistent with previous results (17, 34), virtually all of the FG nucleoporins were retained within a crude nuclear pellet in the wild-type strain and about half of each population could be solubilized by extraction with a high concentration of salt (S2), the remainder staying with the insoluble pellet fraction (Fig. 5). When MAb 414 (which recognizes predominantly Nsp1p and Nup2p) was used to probe equal amounts of each fraction from the three strains, we found that the overall level of Nup2p was reduced in the *nle3*Δ mutant relative to that of the isogenic control, particularly in the insoluble pellet fraction (Fig. 5A, upper panel). In addition, we found that MAb 414 recognized a prominent polypeptide migrating at ~130 kDa in the *nle3*Δ strain that was barely detectable in wild-type or *yer105*Δ strains. This polypeptide was also present when blots were probed with MAb 306, which recognizes Nup1p, Nup2p, and Nsp1p (Fig. 5A, lower panel). In fact, it comigrated exactly with Nup1p. To definitively assign each of these polypeptides, we next examined the pattern in *nup1*Δ and *nup2*Δ deletion mutants (Fig. 5B). Blots of S2 and pellet fractions from *nup2*Δ strains probed with either MAb 306 or 414 confirmed that the lower of the three bands is in fact Nup2p, because it was absent in the *nup2*Δ deletion. Similarly, the uppermost band present in the S2 fraction of wild-type cells was absent in the *nup1*Δ mutant, confirming that this band corresponds to Nup1p. However, when we examined the pellet fraction from *nup1*Δ cells, we observed that a 130-kDa band was not only present but was overexpressed in a manner similar to that observed with the *nle3*Δ strain. Furthermore, this protein reacted strongly with MAb 414 whereas Nup1p does not. We conclude that there is another protein containing XFXFG repeats which comigrates with Nup1p and is increased in abundance when either *NUP1* or *NLE3* is disrupted. Although the presence of the comigrating polypeptide precluded us from determining the relative amounts of Nup1p in pellet fractions from each of the three strains, we were able to compare amounts in the salt extracts because, of these two proteins, only Nup1p is extractable. We consistently observed that negligible amounts of Nup1p were recovered in the S2 fraction from either *nle3*Δ or *yer105*Δ strains (Fig. 5A). Thus, both Nup1p and Nup2p appear to be less tightly associated with the NPC in the *nle3*Δ mutant whereas the level of a fourth XFXFG protein is increased. These changes are also reflected in the total amount of each protein recovered, in that we did not observe concomitant changes in the amount of protein in another fraction. This is similar to our previous observations with *nup1* mutants that are not correctly localized (10), and we think it most likely that FG nucleoporins are rapidly degraded if they are not assembled into the NPC. We also assayed the fractionation of the GLFG nucleoporins recognized by MAb 192. In this case, we did not detect consistent differences between fractionation in either of the two deletion mutants and that in wild-type cells (data not shown).

TABLE 2. Segregation data from genetic crosses between nucleoporin mutants

Genotype of parent 1 strain	Results for parent 2 strain with genotype:					
	nle3-Δ1::HIS3			*yer105::URA3*		
	Marker	No. of spores recovered that were:		Marker	No. of spores recovered that were:	
		Viable	Inviable[a]		Viable	Inviable
nup1-Δ1::LEU2	Leu^+	81	2	Leu^+	14	5
	His^+	72	13	His^+	19	0
	Leu^+ His^+	2[b]	68	Leu^+ His^+	0	10
nup2-Δ1::TRP1	Trp^+	32	0	Trp^+	14	1
	His^+	16	16	Ura^+	14	1
	Trp^+ His^+	0	45	Trp^+ Ura^+	17	1
nup100::URA3	Ura^+	11	1			
	His^+	11	2	Not tested		
	Ura^+ His^+	10	1			
nle4-1[ts]	Ts^-	24	13	Ts^-	15	0
	His^+	36	0	Ura^+	15	0
	Ts^- His^+	0	28	Ts^- Ura^+	0	20
nle3-Δ1::HIS3				His^+	17	2
				Ura^+	15	0
				His^+ Ura^+	0	20
srp1-175::LEU2	Leu^+	6	0	Leu^+	11	0
srp1-Δ2::KAN	His^+	8	0	Ura^+	10	0
	Leu^+ Kan^r	9	0	Leu^+ Kan^r	9	0
	Leu^+ His^+	5	0	Leu^+ Ura^+	9	0
	Leu^+ Kan^r His^+	0	1	Leu^+ Kan^r Ura^+	9	0
srp1-183::LEU2	Leu^+	3	0	Leu^+	7	0
srp1-Δ2::KAN	His^+	8	0	Ura^+	9	0
	Leu^+ Kan^r	9	0	Leu^+ Kan^r	10	0
	Leu^+ His^+	4	0	Leu^+ His^+	9	0
	Leu^+ Kan^r His^+	0	6	Leu^+ Kan^r His^+	10	1
srp1-31 CEN TRP1	Trp^+	12	ND[c]	Trp^+	5	ND[c]
srp1-Δ2::KAN	His^+	15		Ura^+	9	
	Trp^+ Kan^r	9		Trp^+ Kan^r	5	
	Trp^+ Kan^r His^+	0		Trp^+ Kan^r Ura^+	4	
	Trp^+ His^+	0		Trp^+ Ura^+	6	
srp1-49 CEN TRP1	Trp^+	11	ND[c]	Trp^+	16	ND[c]
srp1-Δ2::KAN	His^+	18		Ura^+	9	
	Trp^+ Kan^r	10		Trp^+ Kan^r	28	
	Trp^+ Kan^r His^+	0		Trp^+ Kan^r Ura^+	21	
	Trp^+ His^+	0		Trp^+ Ura^+	27	

[a] The genotypes of inviable spores were inferred where possible. The numbers in this column are lower than actual numbers because genotypes could not always be determined.

[b] These spores did not have the temperature-sensitive phenotype of *nup1Δ* and probably resulted from gene conversion at *LEU2*.

[c] Because *srp1-31* and *srp1-49* alleles are carried on plasmids that do not necessarily segregate 2:2, the complete genotypes of inviable spores could not be inferred in this cross. ND, not determined.

To further localize the XFXFG nucleoporins in the *nle3Δ* mutant, we performed immunofluorescence with anti-XFXFG MAb 414. We observed no gross differences in staining intensities or overall localizations of the XFXFG nucleoporins in *nle3Δ* cells grown at 23°C, compared with those of an isogenic wild-type control (Fig. 6). Furthermore, we saw no indication of defects in nuclear envelope architecture, such as clustering of NPCs or misshapen nuclear envelopes, which are observed in many nucleoporin deletion backgrounds (23, 27, 33, 60, 61). This result suggests that the nuclear envelope and NPCs are at least grossly intact in the *nle3Δ* mutant. Furthermore, MAb 414 specifically decorates the NPC in the *nle3Δ* mutant just as it does in wild-type cells, suggesting that the unidentified XFXFG protein overexpressed in this strain is likely to be a nucleoporin. Definitive proof of this awaits the identification of this protein.

Nle3p associates with the amino-terminal domain of Nup1p. We next wanted to investigate whether Nle3p might function directly to anchor Nup1p within the NPC. To address this issue, we first determined whether fractionation of Nle3p itself is consistent with such a role. We integrated a c-*myc*-tagged version of *NLE3* by transforming *nle3Δ* strain JRY9 with a *LEU2*-integrating plasmid containing *NLE3[myc]* (pJP15; see Materials and Methods). When blots containing S1, S2, and

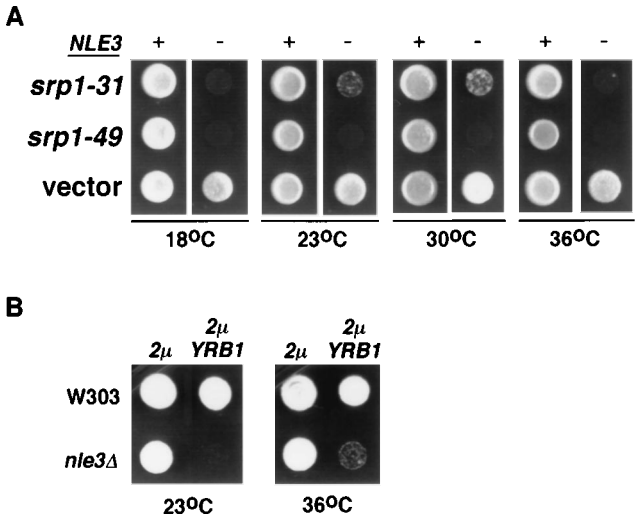

FIG. 4. Genetic interactions between *NLE3* and genes encoding soluble transport factors. (A) *CEN TRP1* plasmids containing temperature-sensitive *srp1-31* and *srp1-49* alleles, or plasmid alone, were transformed into strain JPY21 (*nle3-Δ1::HIS3* [*NLE3 CEN URA3*]). Transformants were then plated to either 5FOA without Trp (−) or medium without Trp (+). Duplicate plates were grown at each of the indicated temperatures. (B) Isogenic strains LDY433 (*NLE3*; top) and JPY21 (*nle3-Δ1::HIS3*; bottom) were transformed either with *YRB1* carried on a 2μm *URA3* plasmid (pLDB187) or with empty plasmid. Transformants were grown at 36°C and then plated to duplicate plates for incubation at the indicated temperatures.

pellet fractions from this strain (JPY41) were probed with anti-Myc MAb 9E10, we found that Nle3p (which migrates at ~195 kDa) partitioned between the S2 and pellet fractions in a manner very similar to that of the FG nucleoporins (Fig. 7).

We next investigated whether the Nle3 protein extracted by 1 M salt could bind to Nup1p. To this end, we utilized GST fusion proteins containing each of the three domains of Nup1p, as well as progressive truncations of the amino-terminal domain which correspond to the *nup1-8*, *nup1-9*, and *nup1-15* mutants. These fusion proteins were used to precipitate interacting proteins from the S2 fraction of cells containing the tagged Nle3p construct, and then Western blotting (immunoblotting) with anti-Myc MAb 9E10 was performed (Fig. 8). We found that the amino-terminal Nup1::GST fusion specifically precipitated Nle3p. Furthermore, when truncations of the amino-terminal GST fusion which correspond to the *nup1-8*, *nup1-15*, and *nup1-9* mutants were made, the strength of the interaction progressively weakened. The abilities of the different amino-terminal fusion proteins to bind to Nle3p correspond very closely to the amount of each truncated Nup1p protein previously shown to be associated with the postnuclear pellet fraction (10). Thus, Nle3p associates specifically with the domain of Nup1p implicated previously in its localization.

DISCUSSION

In this study we have identified two homologous yeast proteins, Nle3p and Yer105p, both of which are similar to the mammalian nucleoporin Nup155. Although neither is essential in and of itself, together these proteins are required for viability. Deletion of *NLE3*, and to a lesser extent *YER105*, exacerbates the phenotypes of a broad spectrum of mutants that affect translocation across the NPC, including the FG nucleoporins themselves as well as soluble factors that mediate docking and translocation.

Several lines of evidence suggest that Nle3p mediates the assembly of Nup1p within the NPC. *nle3-1* is one of only two *nle* mutants that differentiate between the progressive *NUP1* amino-terminal truncation mutants *nup1-8* and *nup1-15*. Strains carrying either of these *nup1* alleles grow quite well even though both are missing a considerable amount of the amino-terminal domain. However, when assayed biochemically

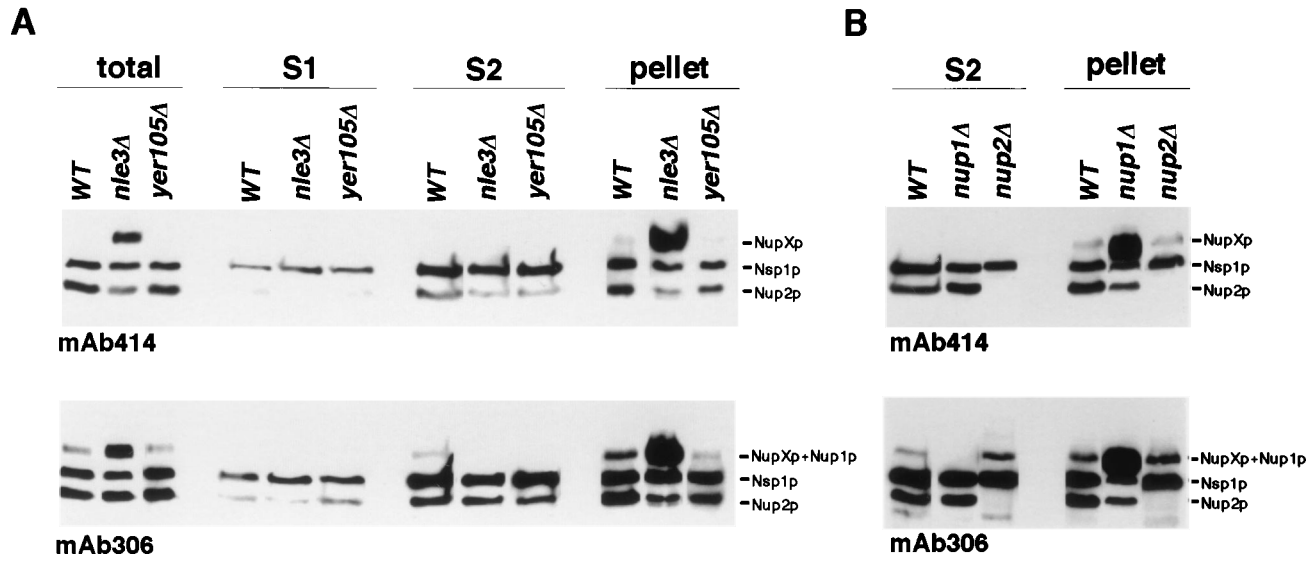

FIG. 5. Alterations in the stoichiometry of XFXFG nucleoporins in *nle3*Δ and *yer105*Δ strains. (A) Spheroplasts from isogenic strains LDY433 (*WT*), JPY21 (*nle3*Δ), and JPY45 (*yer105*Δ) were lysed and fractionated by low-speed centrifugation into soluble (S1) and postnuclear pellet fractions. The pellets were then extracted in buffer containing 0.5 M NaCl, which releases about half of the FG nucleoporins. Solubilized proteins were collected in another low-speed centrifugation supernatant (S2), and the remaining pellet was solubilized with SDS sample buffer. Fractions were subjected to SDS-polyacrylamide gel electrophoresis, loading 0.5 OD_{600} equivalents of total spheroplast lysate per lane and 2 OD_{600} equivalents of each subcellular fraction per lane. Coomassie blue staining was used to confirm that equal amounts of protein were present in fractions from each strain. Separated proteins were blotted to nitrocellulose, and blots were probed with either MAb 414 (top panel) or MAb 306 (bottom panel). (B) Spheroplasts from isogenic strains LDY433 (*WT*), LDY626 (*nup2*Δ), and LDY461 (*nup1*Δ) were analyzed exactly as described for the gels in panel A.

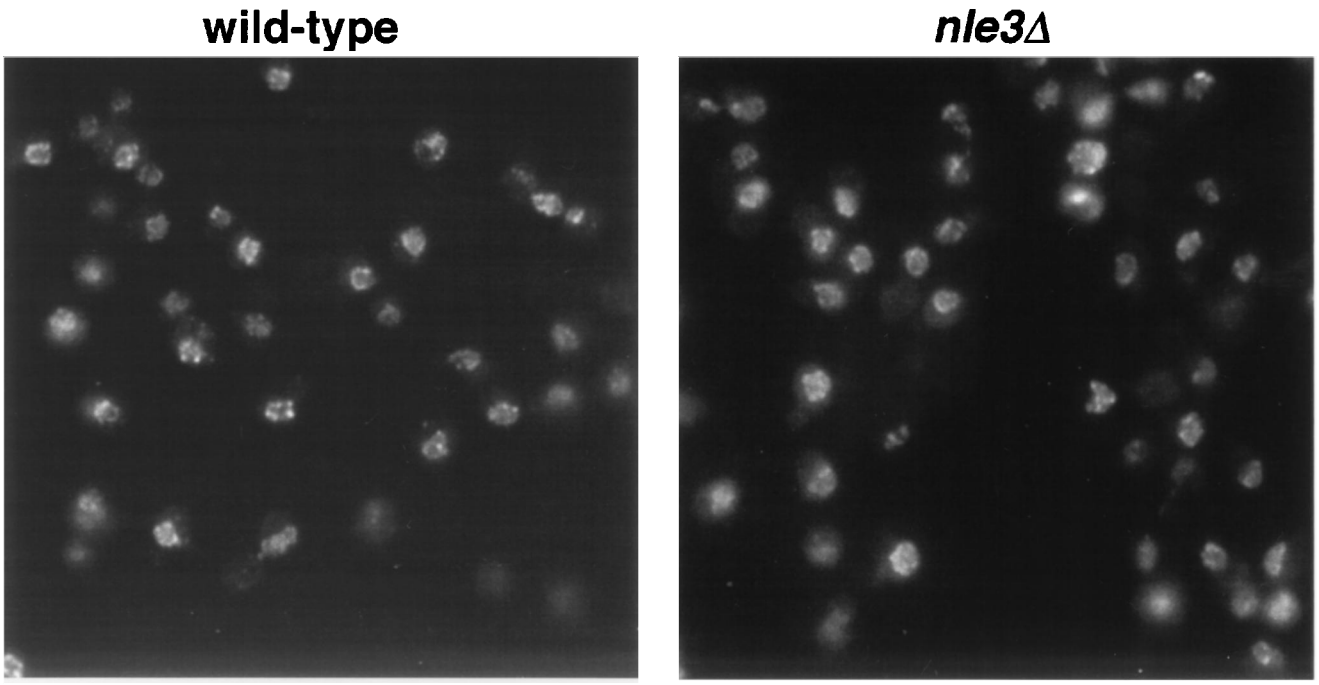

FIG. 6. Localization of XFXFG nucleoporins in the *nle3*Δ mutant. Anti-XFXFG antibody 414 was used for indirect immunofluorescence on wild-type yeast strain LDY433 (left panel) and on an otherwise isogenic *nle3*Δ mutant strain, JPY21 (right panel). Cells were grown at 25°C prior to fixation, and spheroplasting was performed as described by Wente et al. (62). MAb 414 ascites fluid was used at a dilution of 1:200. Secondary DTAF-labelled anti-mouse antibody (Jackson Immunoresearch Labs) was used at a dilution of 1:100.

they are quite different. The amount of Nup1-8p in the nuclear pellet fraction is close to that of wild-type cells, whereas Nup1-15p is barely detectable in this fraction. Removal of just 20 amino acids more (*nup1-9*) renders Nup1p completely nonfunctional (10). These results suggest not only that the amino-terminal domain of Nup1p is required for its localization to the NPC, but also that there is a threshold amount of Nup1p required at the NPC for normal function. Carboxyl-terminal truncations behave differently in that even a small truncation confers a severe growth defect, although the mutant protein appears to be correctly localized, suggesting that the carboxyl-terminal domain is required for Nup1p function within the NPC. In agreement with this, we have found that the binding site for Srp1p lies within the degenerate FGXN repeats located in the carboxyl-terminal domain of Nup1p (5a). Consistent with the genetic predictions, we have shown that Nle3p can be precipitated specifically by GST fusions containing the amino terminus of Nup1p. Furthermore, we observed a progressive loss of binding to amino truncations that remove the same residues missing in the *nup1-8*, *nup1-15*, and *nup1-9* truncation mutants. Thus, Nle3p binding correlates well with the localization of Nup1p and with the allele-specific interactions between *nle3-1* and *nup1* mutants. Lastly, our results show that Nup1p is mislocalized in the absence of Nle3p.

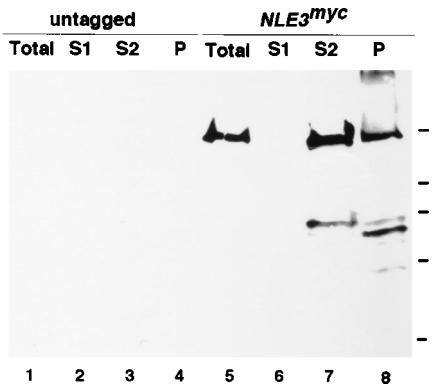

FIG. 7. Fractionation of epitope-tagged *NLE3*. Spheroplasts from yeast strain JPY41 (*nle3-Δ1::HIS3* [*NLE3*^{myc} *LEU2*]) were spheroplasted and fractionated exactly as described in the legend to Fig. 4, along with an untagged control strain. Blots of cell fractions were probed with MAb 9E10, which recognizes the c-*myc* tag. Lanes 1 to 4, untagged cell fractions; lanes 5 to 8: tagged cell fractions. Molecular mass standards marked at the right are (from top to bottom) 200, 116, 97, 66, and 45 kDa. P, pellet fraction.

At present, we do not know the precise function of Nle3p. Our observation that Nle3p can be precipitated specifically by the region of Nup1p required for its localization suggests that Nle3p may play a direct role in tethering specific nucleoporins. However, we have not determined whether Nle3p binds directly to Nup1p, and it remains possible that an entire NPC subunit is precipitated by the Nup1::GST fusion protein. If so, then Nle3p could play a role in subunit assembly rather than as a specific anchor for Nup1p. It is unlikely that deletion of *NLE3* has profound effects on the assembly of the NPC as a whole, however, because immunofluorescence staining with anti-XFXFG MAb 414 was not visibly altered in the *nle3*Δ mutant. Furthermore, both Nle3p and the FG nucleoporins are extracted under conditions that leave the core structures of the NPC intact. Whatever its exact role, the identification of *NLE3* should now allow us to investigate in more detail the process of NPC assembly, about which very little is known at present.

Because *NUP1* is not essential in the strain background employed to isolate the *nle* mutants, Nup1p localization cannot be the sole function of Nle3p. Indeed, the results of our frac-

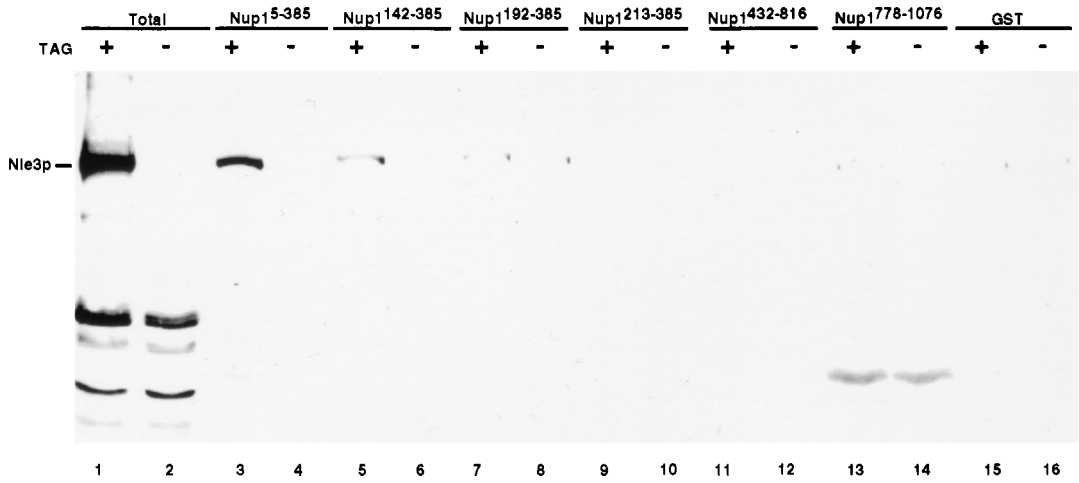

FIG. 8. Association of Nle3p with the amino terminus of Nup1p. Strain LDY433 was transformed with either pJP15 (*NLE3*^{myc}, odd-numbered lanes) or empty vector (even-numbered lanes). Cells were spheroplasted and lysed after growth at 23°C, and the crude nuclear pellet was extracted with 1 M salt (see Materials and Methods). Salt extracts were then diluted and incubated with glutathione-Sepharose adsorbed with equal amounts of GST fusion proteins containing the indicated amino acid residues of Nup1p (lanes 3 to 14). GST alone was used as a negative control (lanes 15 and 16). Total extract (one-fifth of the amount used for precipitation) was run in parallel (lanes 1 and 2). Samples were electrophoresed and blotted to nitrocellulose, and the blots were then probed with anti c-*myc* MAb 9E10 ascites fluid. Note that a minor protein migrating slightly faster than Nle3p is also precipitated by some of the GST fusion proteins, as well as by GST alone. Although this protein is recognized by MAb 9E10, it is not Nle3p because it is present in both tagged and untagged samples.

tionation analysis suggest that both Nup1p and Nup2p is mislocalized in the *nle3*Δ mutant. This observation provides an explanation as to why *nle3* is lethal if either *NUP1* or *NUP2* is deleted. Genetic evidence suggests that Nup1p and Nup2p function on separate, redundant pathways for translocation of karyopherin-substrate complexes across the NPC (27a). So far, only mutations in karyopherin α (*srp1*) are synthetically lethal with both *nup1*Δ and *nup2*Δ, presumably because karyopherin interactions are important for the function of both pathways. We suggest that in the absence of fully functional Nle3p, the amount of Nup1p and Nup2p correctly localized within the NPC is just sufficient for a threshold level of translocation, provided that both pathways function. If so, then completely blocking one pathway by deleting either Nup1p or Nup2p would further slow translocation enough to cause lethality.

In addition to a decrease in Nup1p and Nup2p levels in the *nle3*Δ mutant, we observed a concomitant increase in the level of an unidentified polypeptide that is recognized by anti-XFXFG MAbs 414 and 306. While the identity of the other polypeptide remains to be determined, it may be another member of the XFXFG nucleoporin family, perhaps one which normally functions in a manner analogous to that of Nup1p on the opposite pathway. Although the increased amount of this protein could reflect a change in its expression levels in the *nle3*Δ mutant, we think it more likely that the protein is preferentially stabilized, perhaps because it is misincorporated at sites normally occupied by Nup1p. This view is supported by the observation that a protein of the same molecular weight, and which is recognized by MAbs 414 and 306, is also overexpressed in the *nup1*Δ mutant. While the mechanism that regulates the stoichiometry of the various FG nucleoporins within the NPC is currently unclear, we note that overexpression of many of the FG nucleoporins is lethal (17, 34, 60), suggesting that misincorporation can occur if the balance of different nucleoporins is greatly perturbed. Another alternative is that this protein is a modified form of an FG nucleoporin. In metazoan cells the FG nucleoporins are modified by O-linked *N*-acetylglucosamine, although this has not been demonstrated in yeast cells. Also, one of the yeast GLFG nucleoporins, Nup145p, appears to be proteolyzed to a smaller form immediately after synthesis (20, 61). Although the protein we observed is not a stabilized form of Nup145p, because it is not recognized by anti-GLFG MAb 192, it is possible that a novel XFXFG nucleoporin normally undergoes the same type of cleavage process but is stabilized in the *nle3*Δ mutant.

The possibility of misincorporation of FG nucleoporins suggests an alternative, and not necessarily mutually exclusive, explanation for the synthetic phenotypes observed with the *nle3* deletion mutant. If specific sublocalization of nucleoporins is important for directed movement of karyopherin across the NPC, then substituting one Nup for another could have an adverse effect on translocation. According to this view, at least some of the detrimental effects of losing Nle3p have less to do with the partial loss of Nup1p and Nup2p than with the fact that they are replaced with an inappropriate FG nucleoporin. In this regard, the dominant lethality observed upon combining *nle3*Δ with mutations in the *arm* repeats of Srp1p is particularly interesting. The profound dominance of these two normally recessive alleles of *srp1* could be explained if the defective karyopherin complex cannot dissociate from an FG nucleoporin that is present out of sequence along the translocation pathway, thus leading to a lethal block to translocation. Another alternative is that these *srp1* alleles titrate an interacting protein (such as karyopherin β), thus leading to a subtle dominant defect in nuclear import that is not apparent unless it is combined with another mutation affecting NPC function. In this case, the dominance may not be specifically related to Nle3p function.

Because the *yer105*Δ mutant has generally much less pronounced phenotypes than *nle3*Δ, both biochemically and genetically, *YER105* may simply encode a minor homolog of Nle3p. An alternative possibility is that Nle3p and Yer105p are responsible for assembling different FG nucleoporins. According to this view, the difference in phenotypes between the two disruptions reflects the particular requirements for a given set of nucleoporins. If so, the synthetic lethality between *nle3*Δ and *yer105*Δ may reflect additive effects rather than true redundancy. We also note that mammalian Nup155 complements

neither the cold-sensitive phenotype of *nle3*Δ itself nor the synthetic phenotype observed between *nup1*Δ and *nle3*Δ or *yer105*Δ and *nle3*Δ. While it is quite possible that the yeast and mammalian proteins are in fact functional homologs that have simply diverged enough to preclude cross-species complementation, an alternative view is that there exists a large family of similar proteins, perhaps each responsible for localizing a distinct set of FG nucleoporins. If so, the exact yeast homolog of Nup155 may remain to be identified.

After submission of this work, Aitchison et al. (1) showed that both Nle3p and Yer107p, which they refer to as Nup170p and Nup157p, are localized to the NPC. In accordance with accepted nomenclature, we will hereafter refer to *NLE3* as *NUP170* and *YER105* as *NUP157*.

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