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Npp106p, a *Schizosaccharomyces pombe* Nucleoporin Similar to *Saccharomyces cerevisiae* Nic96p, Functionally Interacts with Rae1p in mRNA Export

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To identify components of the mRNA export machinery in *Schizosaccharomyces pombe*, a screen was developed to identify mutations that were synthetically lethal with the conditional mRNA export allele *rae1-167*. Mutations defining three complementation groups were isolated, and here we report the characterization of *npp106* (for nuclear pore protein of 106 kDa). This gene encodes a predicted protein that has significant similarity to the Nic96p nucleoporin of *Saccharomyces cerevisiae*. Consistent with Npp106p being a nucleoporin, a functional green fluorescent protein (GFP)-tagged Npp106p localized to the nuclear periphery. In contrast to *NIC96*, the *npp106* gene is not essential. Moreover, a Δ*npp106* mutant did not show cytoplasmic mislocalization of a simian virus 40 nuclear localization signal-GFP-LacZ reporter protein, and a fraction of cells had accumulation of poly(A)$^+$ RNA in the nucleus. A consequence of the synthetic lethality between *rae1-167* and *npp106-1* was the accumulation of poly(A)$^+$ RNA in the nucleus when cells were grown under synthetic lethal conditions. In addition to *npp106-1*, which is a nonsense mutation that truncates the protein at amino acid 292, the Δ*npp106* mutation was synthetically lethal with *rae1-167*, suggesting that the synthetic lethality is a consequence of the loss of a function of *npp106*. We further demonstrate that a region between amino acids 74 and 348 of Npp106p is required for complementation of the synthetic lethality. These results uncover a potential direct or indirect involvement of Npp106p in mRNA export.

Nuclear pores mediate the transport of proteins and RNA between the nucleus and cytoplasm in a bidirectional fashion (14, 28, 29, 43, 47, 59, 61). These molecules are transported through the nuclear pore complex (NPC) by an active process that is saturable, energy dependent, and signal specific. Over the past few years, many of the essential components of the protein import machinery have been identified. These include the importin α/β complex, which recognizes nuclear localization signal (NLS)-bearing proteins, as well as components of the Ran-GTPase switch system, which provides the energy for transport (28, 29, 47, 61). Moreover, these factors interact with proteins of the NPC, and these interactions may be functionally important. Progress has also been made in identifying the factors required for mRNA export. In particular, studies with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have identified nuclear pore and nuclear pore-associated proteins (1, 18, 30, 32, 36, 39, 50, 66, 70, 71) as well as some non-NPC proteins that are required for mRNA export (6, 7, 9, 13, 21, 38, 45, 46, 51, 57, 58, 68). It has been proposed that the export of mRNA is mediated by carrier proteins (19, 20, 23, 25, 37, 42, 49, 63, 69, 71, 72) that bind the mRNA and through their interactions with the NPC export the RNA through the pore.

Interaction between the carrier and the NPC is thought to be mediated by a domain on the carrier referred to as the nuclear export signal (NES) (26). So far, two NES motifs have been described. One type has been found in the RNA binding proteins human immunodeficiency virus Rev (19, 23, 25, 71) and TFIIIA (24), as well as in an mRNA export factor (Gle1p [58]), the importin β of yeast (40), and the protein kinase inhibitor of cyclic AMP-dependent protein kinase (72). This NES is a leucine-rich 8- to 10-amino-acid sequence that is required for the export of these proteins from the nucleus. This leucine-rich sequence has been shown to interact with a nucleoporin-like protein, Rip1p/Rab1p in human cells (25) and with Nup42/Rip1p in *S. cerevisiae* (71). This protein is a member of the FG repeat protein family. It has been proposed that the interaction between the NES and Rip1p/Nup42p is functionally important in targeting the mRNA-protein complex to the NPC for export into the cytoplasm (25, 71). Also in yeast, the human immunodeficiency virus type 1 Rev and Gle1p NESs interact with the GLFG repeat protein Nup100p (58, 71). A second type of NES, M9, has been identified in hnRNP-A1 (55). It is a 38-amino-acid sequence that contains signals for protein import and export. No mutation within this region that affects only one transport function has been isolated. It interacts with transportin, a protein related to importin β, and is sufficient to signal import of a heterologous protein by a mechanism that is independent of the classical NLS-dependent pathway mediated by the importin α/β complex (64). A transportin homolog in *S. cerevisiae*, Kap104p, has also been shown to be required for the import of an mRNA binding protein, Nab2p, but the required import signal has not been identified (2). It is unknown whether Kap104p also mediates the export of M9-bearing proteins in *S. cerevisiae.*

Several nucleoporins in *S. cerevisiae* that may participate in mRNA export from the nucleus have been identified. These can be classified according to the presence of repeated sequences: Nup1p is a member of the FXFG repeat class (66); Nup159p is a member of the FG repeat class (48); Nup49p, Nup116p, and Nup145p are members of the GLFG repeat class (16, 18, 73, 74, 76); and Nup82p, Nup84p, Nup85p, Nup120p, and Nup133p are nonrepeat nucleoporins (1, 16, 27,

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TABLE 1. Strains

Strain	Genotype	Source or reference
972	*h*$^-$	
JBP16	*h*$^-$ *leu1-32 ura4-D18*	11
JBP174	*h*$^+$ *leu1-32 ura4-D18*	This study
JBP167	*h*$^-$ *leu1-32 ura4-D18 rae1-167*	This study
JBP168	*h*$^-$ *leu1-32 ura4-D18 rae1-167*/pREP81X-*rae1*$^+$	This study
SL64	*h*$^-$ *leu1-32 ura4-D18 rae1-167 npp106-1*/pREP81X-*rae1*$^+$	This study
KGY634	*h*$^+$ *ade6-704 his3-237 leu1-32 ura4-D18*	K. Gould
JBP169	*h*$^-$ *leu1-32 ura4-D18 rae1-167 npp106-1*::pIA2/pREP81X-*rae1*$^+$	This study
JBP170	*h*$^-$ *leu1-32 ura4-D18* Δ*npp106*::*ura4*	This study
JBP171	*h*$^-$ *leu1-32 ura4-D18 npp106-1*	This study
SP826	*h*$^+$/*h*$^+$ *leu1-32*/*leu1-32 ura4-D18*/*ura4-D18 ade6-210*/*ade6-216*	53
JBP173	*h*$^+$/*h*$^+$ *leu1-32*/*leu1-32 ura4-D18*/*ura4-D18 ade6-210*/*ade6-216* Δ*npp106*::*ura4*/*npp106*	This study
ptr3-1 mutant	*h*$^-$ *ptr3-1 leu1-32 ura4-D18*	9

32, 36, 39, 50, 62, 67, 70). A large number of genetic interactions among them have been identified through synthetic lethal screens (14, 15). These genetic interactions may reflect either direct physical interaction or redundant functions within the pore. It is likely that some of these nucleoporins interact with carrier proteins and other factors to mediate mRNA export from the nucleus.

We have been studying the role of Rae1p in mRNA export from the nucleus in the fission yeast *S. pombe*. The temperature-sensitive *rae1-1* mutant rapidly accumulates mRNA in the nucleus upon a shift to a restrictive temperature (11). The primary sequence of Rae1p is highly conserved throughout evolution. Indeed, the human homolog can complement the growth and mRNA export defects of the *rae1-1* mutant, suggesting that its function is also conserved. Recently, a homolog in *S. cerevisiae*, Gle2p, has been described (57). A conditional mutation, *gle2-1*, confers an mRNA export defect at the restrictive temperature and is synthetically lethal with a Δ*nup100* mutation. While an interaction between Gle2p and Nup42p/Rip1p was detected by using a two-hybrid system, no interaction between Gle2p and Nup100p was detected. Recently, Gle2p has been isolated in a subcomplex containing Nup116p, a GLFG repeat protein (40). Both Rae1p and Gle2p localize to the nuclear periphery (57, 75), and Gle2p can be found in a fraction enriched for nuclear pore proteins (57). This is consistent with the model that Rae1p/Gle2p functions at the nuclear pore to export mRNA either directly as a component of the export machinery or indirectly by interacting with other NPC components.

To identify components of the mRNA export machinery that genetically interact with *rae1*, we developed a synthetic lethal screen to look for mutants that require *rae1*$^+$ expression for growth. Here we describe the genetic interaction between *rae1* and *npp106*, which encodes a nucleoporin with similarity to a nonrepeat nucleoporin of *S. cerevisiae*, Nic96p (31).

MATERIALS AND METHODS

Strains and culture. Strains used in the study are listed in Table 1. The basic genetic techniques used have been described previously (4, 56). The *rae1-167* mutant was isolated after nitrosoguanidine mutagenesis of the original *rae1-1* mutant (11). Random spore analysis demonstrated that it contained an intragenic suppressor (H286R). The *rae1-167* mutant was backcrossed four times against JBP16, and a tetrad analysis yielded a 2:2 segregation of the *rae1-167* allele (20 tetrads). The media used have been described previously (4, 56). Appropriately supplemented EMM medium was used to express genes from the *nmt* promoter (54). The *nmt* promoter was repressed by the addition of 0.5 μM thiamine in EMM medium.

Synthetic lethal screen. JBP168 was mutagenized with 2% ethyl methanesulfonate (EMS) for 3.5 h, with a survival of 30%. After inactivation of EMS with sodium thiosulfate, cells were plated onto EMM agar plates containing uracil in absence of thiamine. Colonies were replica plated on to EMM agar plates containing phloxin B in the presence (*rae1*$^+$ repressed) and absence (*rae1*$^+$ expressed) of thiamine at 27°C. Synthetic lethal mutants were identified as those that were red in presence of thiamine (dead cells take up the red phloxin B) but pink in absence of thiamine. Approximately 500,000 colonies were screened, and six potential synthetic lethal mutants were isolated. These mutants were crossed with the *h*$^+$ strain KGY634, and random spore analysis was performed. Spores were plated on EMM medium without leucine, thereby selecting for the pREP81X-*rae1*$^+$ plasmid, and lacking thiamine. Colonies were replica plated onto EMM plates containing phloxin B with and without thiamine and incubated at 27 and 34°C. Colonies that were red at 27°C in the presence of thiamine were scored as having *rae1-167* and the synthetic lethal mutation. Colonies that were pink at 27°C and red at 34°C in the presence of thiamine were scored as having *rae1-167* without the synthetic lethal mutation. Colonies that were pink at 34°C in the presence of thiamine were scored as either wild type or *rae1*$^+$ with the synthetic lethal mutation. Respective *h*$^+$ synthetic lethal strains were then crossed with the *h*$^+$ synthetic lethal strains. Spores were plated on appropriately supplemented EMM medium lacking thiamine, and the colonies were replica plated onto EMM plates containing phloxin B and thiamine at 27°C. The synthetic lethal mutations were judged to be in the same complementation group if all of the colonies were red. The mutations were judged to be in different complementation groups if there were both red and pink colonies.

Plasmid constructions. The pREP81-*rae1*$^+$ vector was constructed by filling in the ends of a *Not*I fragment carrying the *rae1* cDNA with DNA polymerase Klenow fragment (11) and ligating it into the *Sma*I site of pREP81X (22). The cDNA clone was isolated from a λZAP-II library (a gift of M. Wigler) by plaque hybridization with, as a probe, a fragment of p64-3 from the *Xba*I site upstream of the *npp106* open reading frame to a *Xba*I site in the multicloning site downstream of *npp106*. In vivo excision of pBluescript phagemid from the λZAP-II cDNA clones was performed by using the ExAssit interference-resistant helper phage with the SOLR strain according to instructions provided by Stratagene. p64-3sp was generated by deletion of a fragment from the *Sph*I site in the multicloning site to the *Sph*I site upstream of *npp106*. pDA2 was constructed by inserting the fragments from the *Mlu*I site in *npp106* to the *Bam*HI site in the multicloning site of the cDNA plasmid and the *Sph*I-*Mlu*I fragment from p64-3sp into pDW232 cut with *Sph*I and *Bam*HI. pDA4 and pDA7 were constructed by first generating a PCR product that inserted a *Bam*HI site downstream of termination codons at the positions indicated in Fig. 1A, with pDA2 as a template. For both plasmids the upstream primer was an oligonucleotide spanning the *Sph*I site (underlined), ATCGCATGCCTGGCGCCGCTCCTGG. Thedownstream primers contained the *Bam*HI site (underlined) and termination codon (double underlined) GCGGGATCCTTAATCAATCAATGATAAAAACTGG for pDA4 and (CTAGGATCCTATGCATCGAAAAGTTGAAT for pDA7). The *Sph*I-*Bam*HI fragments from these PCR products were then cloned into pDW232 cut with *Sph*I and *Bam*HI. For all of the constructs described, the regions that were derived from the PCR products were sequenced to ensure that no new mutations were introduced.

pDA8, pDA9, and pDA10 were constructed in the following manner. PCR products were generated from the *Sph*I site upstream of *npp106* to a *Pst*I site inserted just downstream of the indicated codons for each deletion. For the 5′ PCR products, the upstream primer contained the *Sph*I site (underlined) (ATC GCATGCCTGGCGCCGCTCCTGG) and the downstream primers contained the *Pst*I site (underlined) (CTACTGCAGCGATATCACCGTCACGCGTC for pDA8, CTACTGCAGCCTAATCCGTGCCTCATTT for pDA9, and TCTCTG CAGATCCGATATAGAACTTTCTA for pDA10). For the 3′ PCR products, a downstream primer beyond the *Hinc*II site was used (CGTCGTTCTGATTTA CACGG), and the upstream primers contained a *Pst*I site (CACCTGCAGCG ACTAATGAAAAATAATGG for pDA8, CACCTGCAGTTATCATTGATTG ATCTGC for pDA9, and CACCTGCAGGTTTTATTGACGCGTGACGG for pDA10). The respective *Sph*I-*Pst*I upstream fragments and the *Pst*I-*Hinc*II downstream fragments were purified and ligated into pDA2 cut with *Hinc*II and *Sph*I. The resulting deletions place a *Pst*I site and a Leu-Gln at the deletion junction sites.

The green fluorescent protein (GFP) fusion vectors were constructed as follows. The full-length and deletion constructs were cloned into the pZA69U vector (a gift of M. Moser). This plasmid has GFP(S65T) expressed from the wild-type thiamine-repressible *nmt* promoter on pREP3. The GFP(S65T) is fused in frame at the carboxyl terminus to a Gly-Ala linker region followed by a multicloning site. For all constructs an upstream primer inserted a *Sal*I site (underlined) immediately upstream of the initiation codon (double underlined; CATGTCGACATGGAATCCAAGGAAGCCAAAGAG). pZY2 was constructed by first generating a PCR product by using the upstream primer and a downstream primer beyond the *Mlu*I site (GCTTTGATAGCCGTTCTCTA). This product was cut with *Sal*I and *Mlu*I and ligated along with an *Mlu*I-*Bam*HI fragment from pDA2 into pZA69U cut with *Sal*I and *Bam*HI. pZY4 and pZY7

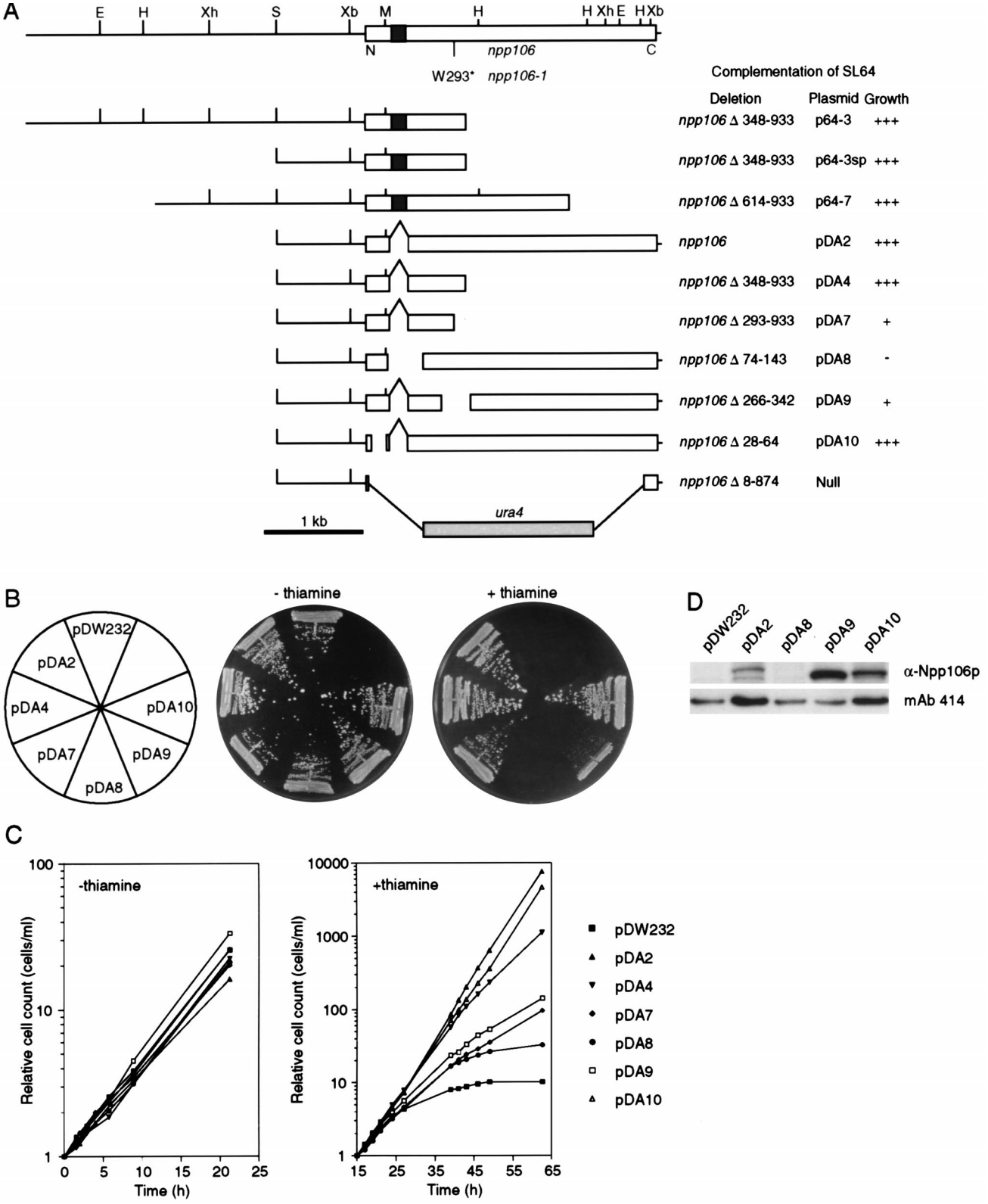

FIG. 1. Complementation of SL64. (A) Ability of deletions to complement the synthetic lethality of SL64. A map of the region surrounding the *npp106* gene is shown. E, *Eco*RI; H, *Hin*dIII; M, *Mlu*I; S, *Sph*I; Xb, *Xba*I; Xh, *Xho*I. The sequence from the *Sph*I site to the poly(A) addition site has been deposited in the GenBank database with the accession number AF000150. Details of the cloning are described in Materials and Methods. The *npp106* open reading frame is denoted by open boxes, and the intron is shown by a shaded box. The location of the nonsense *npp106-1* mutation is noted. The cDNA sequence is denoted by the absence of a shaded box and a line joining the open reading frames. The amino acids removed in the deletions are shown along with the relative growth of SL64 carrying the plasmids. (B) Growth of SL64 carrying different plasmids. The plasmids carried in SL64 are shown on the left. pDW232 is the vector plasmid without an insert. Cells were streaked onto EMM medium with and without thiamine, grown overnight at 27°C, restreaked, and incubated at 27°C for 4 days. (C) Growth of SL64 bearing different plasmids in liquid cultures. Cells were grown in EMM medium without thiamine, and the growth was monitored for 22 h (left panel). Growth of the strains was monitored for 63 h following repression of *rae1*$^+$ expression by the addition of thiamine (right panel). (D) Determination of steady-state levels of Npp106p from crude cell extracts. The upper panel shows the amount of staining for Npp106p for each strain as indicated. The lower panel shows the amount of staining for a protein that reacts to the NPC-specific MAb 414.

were constructed by first generating PCR products by using the upstream primer and the respective primers that inserted a *Bam*HI site and termination codons in pDA4 and pDA7. The respective PCR products were cut with *Sal*I and *Bam*HI and ligated into pZA69U cut with *Sal*I and *Bam*HI. pZY8, pZY9, an pZY10 were constructed by first generating PCR products by using the upstream primer and the respective primers that inserted a *Pst*I site at the deletion junctions in pDA8, pDA9, and pDA10. These products were cut with *Sal*I and *Pst*I and were ligated along with the respective *Pst*I-*Bam*HI fragments from pDA8, pDA9, and pDA10 into pZA69U cut with *Sal*I and *Bam*HI.

The simian virus 40 (SV40) NLS–GFP–β-galactosidase (NLS-GFP-LacZ) construct was a gift from J. Demeter and S. Sazer. It is a derivative of pPS817 (49) in which the SV40 NLS–GFP–β-galactosidase fusion was placed under control of the thiamine-repressible *nmt* promoter in the *S. pombe* expression vector pREP4 (13a, 54). A similar derivative of this fusion was constructed by placing the SV40 NLS–GFP–β-galactosidase fusion in pREP41X, which has the *LEU2* gene as the selectable marker.

Cloning of *npp106* and identification of it as the cognate lethal gene of SL64. The synthetic lethal strain SL64 was transformed with a partial *Sau*3A genomic library cloned into the *Sal*I site of pUR18. Transformants that could grow at 27°C in the presence of thiamine were isolated. Plasmids from these transformants were rescued into *Escherichia coli* and retransformed into SL64. The transformants were sequenced, and analysis was performed by using the Wisconsin Package, version 8, developed by the Genetics Computer Group (Madison, Wis.). The *npp106-1* allele was sequenced by amplification of the full length of the *npp106* coding region by PCR with DNA from SL64 as the template and CTACTAGTATGACTGTAAAT and CGCAGTAAGAAAGGAAGCCA as primers. The products were cloned into TA103 pCR (Invitrogen, San Diego, Calif.). Two independent clones were sequenced, and the *npp106-1* mutation, TGG to TGA at amino acid 293, was the only change present in either clone.

The gene replacement was performed with the pIA2 plasmid. It is a derivative of pDA2 that has the *ars1* element removed by deleting the fragment between the *Sph*I and *Aat*II sites. pIA2 was cut with *Mlu*I and transformed into SL64. Stable Ura^+ transformants that could grow in the presence of thiamine were screened by Southern blotting for strains that had pIA2 integrated at the *npp106* locus. Ura^- colonies were selected on 5-fluoroorotic acid (5-FOA). Strains that had precise excision of pIA2 had either the *rae1-167* phenotype or the SL64 phenotype, demonstrating that the *npp106-1* mutation resides in the region of DNA carried by pIA2.

The Δ*npp106*::*ura4* null strain was constructed as follows. First, a deletion from amino acid 8 to 874 with a *Hin*dIII site inserted was constructed by PCR mutagenesis. For the 5′ PCR product, an oligonucleotide (CAAGCGTAACAC TACCATCC) upstream of the *Xho*I site was used as the upstream primer, and an oligonucleotide that inserted a *Hin*dIII site (underlined) (CCAAGCTTCTT TGGCTTCCTTGGATTC) downstream of amino acid 8 was used as the downstream primer. For the 3′ PCR product, oligonucleotide upstream of the 3′ *Hin*dIII site (TCTGTTAGCAATGTACGAAT) was used as the upstream primer, and an oligonucleotide that introduced a *Sac*I site at the 3′ end of the sequence (CGCGAGCTCTGAACACAACTATCAAGAGG) was used as the downstream primer. The 5′ PCR product cut with *Xho*I and *Hin*dIII and the 3′ product cut with *Hin*dIII and *Sac*I were ligated into pBluescript SK+ vector cut with *Xho*I and *Hin*dIII. Into this *Hin*dIII site a *Hin*dIII fragment carrying *ura4* was inserted. The *Xho*I-*Sac*I fragment was purified and transformed into the SP826 diploid strain. Stable Ura^+ transformants were screened by Southern blotting for the replacement of one of the *npp106* genes. The strain was sporulated, and 20 tetrads were dissected. The tetrads produced four viable spores and a 2:2 segregation of Δ*npp106*::*ura4*. To obtain a Δ*npp106* strain without the *ura4* insert, the Δ*npp106*::*ura4* mutant was transformed with the *Xho*I-*Sac*I fragment without the *ura4* insert, and 5-FOA-resistant cells were obtained. Southern blotting confirmed the loss of the *ura4* marker.

Protein extraction and Western analysis. Cells for Western analysis were grown in appropriately supplemented EMM medium to early log phase. Cells were harvested and washed in a mixture of 100 μg of phenylmethylsulfonyl fluoride per ml, 100 μg of N^{α}-*p*-tosyl-L-lysine chloromethyl ketone per ml, and 100 μg of *N*-tosyl-L-phenylalanine chloromethyl ketone per ml. Cells were broken by vigorous shaking in glass beads in HB buffer (60 mM β-glycerol phosphate, 15 mM *p*-nitrophenyl phosphate, 25 mM MOPS [morpholinepropanesulfonic acid], 1 mM dithiothreitol, 15 mM $MgCl_2$, 0.1 mM sodium metavanadate, 1 mM phenylmethylsulfonyl fluoride, 15 mM EGTA, 1% Triton X-100, 20 μg of leupeptin per ml, 40 μg of aprotinin per ml). Total cell extract was boiled in 5% sodium dodecyl sulfate (SDS) for 5 min, and 20 μg of protein was loaded onto an SDS–10% polyacrylamide gel. Proteins were electroblotted to nitrocellulose, probed with a 1:1,000 dilution of a rabbit polyclonal antibody raised against an Npp106p N-terminal peptide (MESKEAKEKGVNTSDSKGSQ), and detected with an ECL Western blotting kit (Amersham). The membrane was stripped and reprobed with a 1:5,000 dilution of monoclonal antibody (MAb) 414 (Babco, Berkeley, Calif.) (8).

Indirect immunofluorescent localization. Fluorescent in situ hybridization for $poly(A)^+$ RNA was performed as previously described (11), using an oligo-dT(50)-labeled probe followed by a fluorescein isothiocyanate-labeled anti-digoxigenin Fab antibody. Indirect immunofluorescence was performed as described previously (4). To detect nuclear pores, MAb 414 (Babco) was used as the primary antibody (8) and was followed by a fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G antibody. GFP fusion proteins were visualized either in live cells or following fixation for 5 min in 2% formaldehyde and 0.05% glutaraldehyde in phosphate-buffered saline at room temperature. Cells were visualized by using a Zeiss Axiophot microscope with 63× and 100× objectives and were photographed with Kodak Ektachrome 200 slide film. The slides were digitized with a Polaroid Sprint Scan scanner, and the images were processed with the Image Pro Plus software.

Electron microscopy. Cells were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 50 mM potassium phosphate (pH 6.5)–0.5 mM $MgCl_2$ and incubated overnight on ice, and the cell wall was removed by digestion with Zymolyase 100T (4). The cells were postfixed in 2% OsO_4 for 20 min, processed as described previously (12), and embedded in epoxy resin LX112. The cells were washed four times for 15 min each with phosphate-buffered saline, dehydrated in a series of acetone solutions, and embedded in EM bed 812. Thin sections were cut with an LKB-Nova ultramicrotome and stained with uranyl acetate and lead acetate. The samples were observed with a Philips TEM-410 microscope. NPC density was determined by counting the number of pores and measuring the length of the nuclear envelope for 12 nuclei. The length of the nuclear envelope was measured from scanned micrographs by using the Image Pro Plus software package.

Protein-protein interaction. Two-hybrid analysis was performed by using the system described by Durfee et al. (17). Full-length Npp106p and Rae1p were fused to both the Gal4 activation domain ($Gal4_{AD}$) and DNA binding domain ($Gal4_{BD}$) as follows. For Rae1p, a PCR product was generated by using an upstream primer that introduced an *Nco*I site (underlined) at the initiation codon (double underlined) (ATTCCATGGCACTTTTTGGACACTGCTAC) and a downstream primer that introduced a *Bam*HI site (underlined) downstream of the termination codon (double underlined) (CTGGGATCCTTACC TTCCTTTCTTAGGT). This product was cut with *Nco*I and *Bam*HI and ligated into both pASI-CYH2 and pACT2 cut with *Nco*I and *Bam*HI. For Npp106p, a PCR product was generated by using an upstream primer that introduced an *Nco*I site at the initiation codon (CAGCCATGGAATCCAAGGAAGCC) and a primer downstream of the *Mlu*I site (CTCTTTGATACTCCGTTCTCTA). This product was cut with *Nco*I and *Mlu*I and ligated along with the *Mlu*I-*Bam*HI fragment from pDA2 into both pASI-CYH2 and pACT2 cut with *Nco*I and *Bam*HI. *S. cerevisiae* Y190 was cotransformed with these plasmids, and 15 independent transformants were tested. All the transformants gave the same results (a representative of each is shown in Fig. 8). The strains were grown on plates of synthetic medium lacking tryptophan and leucine and assayed for *lacZ* expression on filter paper.

Fusions to glutathione *S*-transferase (GST) were constructed as follows. For GST-Npp106p, the pZY2 vector was cut with *Sal*I and *Not*I and inserted into pGEX-5X-3 vector (Pharmacia) cut with *Sal*I and *Not*I. The GST fusion was expressed in DH5α following induction with IPTG (isopropyl-β-D-thiogalactopyranoside). Npp106p was labeled with [^{35}S]methionine in vitro by transcription from a pET7 vector carrying the *npp106* cDNA from the T7 promoter and translated in rabbit reticulocyte lysates (Promega). Interaction assays of this labeled protein with the GST fusions have been described previously (34).

RESULTS

***rae1-167* synthetic lethal screen.** We used a synthetic lethal screen to identify genes that genetically interact with *rae1*. For this screen, we used the *rae1-167* allele, which retains the original *rae1-1* mutation (G219E) and has a second-site mutation (H286R). This mutation changes a histidine at position 286 to a conserved arginine which is present at that position in all Rae1p homologs from yeast to humans (10). The *rae1-167* mutant has a higher restrictive temperature than the *rae1-1* mutant (32 instead of 25°C) but retains the same phenotype when shifted to the restrictive temperature: rapid accumulation of mRNA in the nucleus and a cell cycle arrest at the G_2/M boundary. To identify mutations that are lethal in combination with *rae1-167*, we used as the parental strain a *rae1-167* mutant expressing $rae1^+$ from the thiamine-repressible *nmt81* promoter on the pREP81X vector. This vector was chosen because when $rae1^+$ expression was repressed by the addition of thiamine, there was no complementation of the *rae1-167* mutant at the restrictive temperature of 34°C (data not shown). This strain was mutagenized with EMS, and approximately 500,000 colonies were screened for the inability to grow at 27°C in the presence of thiamine. Identification of the synthetic lethal mutants was aided by the use of the dye phloxin B, which is taken up by dead cells and causes the colony to become red. Potential synthetic lethal mutants were identified

as red colonies in the presence of thiamine and pink colonies on plates lacking thiamine at 27°C.

Six mutants were isolated, and random spore analysis of a cross between a *rae1*$^+$ strain and these mutants was performed. The spores gave rise to colonies that had the original synthetic lethal phenotype, colonies with the *rae1-167* phenotype, and temperature-resistant colonies. Among the temperature-resistant colonies were those that when crossed to a *rae1-167*/pREP81X-*rae1*$^+$ strain produced spores that gave rise to colonies with synthetic lethal phenotypes identical to those of the original strains. This demonstrated that the synthetic lethal mutations were unlinked to *rae1-167*. Additional crosses among the respective h^+ and h^- synthetic lethal mutants demonstrated that these mutations define three complementation groups: four (SL27, SL40, SL22, and SL8) fell in one complementation group, and SL64 and SL21 each fell in separate complementation groups. In this paper we describe the studies related to SL64.

Cloning of the cognate synthetic lethal gene. The cognate gene carrying the synthetic lethal mutation in SL64 was isolated from a partial *Sau*3A library by functional complementation. Two genomic clones, p64-3 and p64-7, that allowed growth of SL64 at 27°C when *rae1*$^+$ was repressed by the addition of thiamine were isolated (Fig. 1B). Restriction enzyme digestion of the two genomic clones revealed that they had common restriction enzyme fragments. A subclone of p64-3, p64-3sp, that carried a region from the *Sph*I site to the end of the clone was able to functionally complement SL64. This genomic DNA was used as a probe to isolate three full-length cDNA clones. These cDNAs have an open reading frame that can code for a 933-amino-acid protein (Fig. 2). This open reading frame extends well beyond the isolated genomic clones. The cDNA sequence also revealed the presence of an intron near the 5′ end. Sequencing of PCR-generated genomic fragments revealed no additional introns. The cDNA open reading frame was placed under the control of its own promoter, and this plasmid (pDA2) complemented the synthetic lethality of SL64 (Fig. 1) but could not complement the temperature sensitivity of the *rae1-167* mutant.

We next determined whether this open reading frame is the cognate synthetic lethal gene. First, in a marker rescue experiment, the genomic fragment from the *Xba*I site upstream of the open reading frame to a downstream *Xba*I site in the multicloning site in p64-3sp was transformed into SL64. Colonies that were able to grow in the presence of thiamine at 27°C were obtained, indicating that this fragment was able to rescue the synthetic lethality. Second, a gene replacement experiment was performed. The pIA2 plasmid, an *ars1*$^-$ derivative of pDA2 (Fig. 1A), was linearized with *Mlu*I and transformed into SL64. Stable *ura4*$^+$ integrants were isolated, and the integration at the proper site was confirmed by southern analysis. Ura$^-$ colonies were the obtained by selection on 5-FOA. These colonies either had the synthetic lethal phenotype or were able to grow at 27°C in the presence of thiamine. This demonstrated that the synthetic lethal mutation resides in the region of DNA carried by the pDA2 plasmid. Finally, the DNA of the entire open reading frame was amplified by PCR from SL64, and the sequence revealed that there is a single mutation: the DNA encoding the amino acid at position 293 is mutated from a tryptophan codon (TGG) to a translational termination codon (TGA).

The product of the cognate synthetic lethal gene shows similarity to *S. cerevisiae* Nic96p. A BLASTP search (5) of the protein databases revealed that the cognate synthetic lethal protein has significant similarity to a nuclear pore protein of *S. cerevisiae*, Nic96p (31). We will refer to this *S. pombe* gene as *npp106*, for nuclear pore protein with molecular mass of 106 kDa. Two additional predicted proteins with similarities to Npp106p were also identified: one from *Homo sapiens* and another from *Xenopus laevis*. Alignment of these sequences revealed that the similarity among these proteins extends throughout the entire sequence. The Npp106p protein is 28% identical and 52% similar to Nic96p of *S. cerevisiae*, 22% identical and 47% similar to the human protein, and 23% identical and 50% similar to the *Xenopus* protein (Fig. 2). The *Xenopus* protein is 84% identical and 91% similar to the human protein.

The N-terminal region of Nic96p (amino acids 1 to 150) contains three heptad repeats that are required for interaction with a complex containing Nsp1p, Nup57p, and Nup49p and are required for import of an NLS-bearing reporter protein (33). Analysis of all of the proteins similar to Nic96p by using the COILS program (52) predicted a coiled-coil structure surrounding the first heptad repeat identified by Grandi et al. (33). While this program gave low probabilities for the second and third heptad repeat regions in all of the proteins, inspection of the aligned sequences revealed conserved hydrophobic amino acids spaced seven residues apart (Fig. 2). Additionally, there is a conservation surrounding the conditional mutations of *NIC96*, and this region has been implicated in NPC biogenesis (77). Finally, the C-terminal region is well conserved throughout, and this region is required for interaction with Nup188 (60, 77).

The *npp106* gene is not essential. In *S. cerevisiae*, *NIC96* is an essential gene (31). To determine whether *npp106* is essential, a gene disruption, in which nearly all of the *npp106* gene was replaced with *ura4* (Fig. 1A), was constructed in a diploid. Dissection of 20 tetrads showed that all spores formed colonies, and there was a 2:2 segregation of the uracil prototrophy. These results indicate that *npp106* is not an essential gene. Moreover, both the *npp106-1* and Δ*npp106* mutants grew well at all temperatures between 21 and 36°C. Additionally, the Δ*npp106* mutation was synthetically lethal with *rae1-167* (data not shown). This suggests that the synthetic lethality between *npp106-1* and *rae1-167* is a result of the loss of *npp106* function.

Npp106p has a distinct domain required for genetic interaction with *rae1*. To gain a better understanding of the *rae1-npp106* genetic interaction, we determined what sequences within *npp106* were required to complement the synthetic lethality of SL64 (Fig. 1). A multicopy plasmid expressing a protein that is truncated by the *npp106-1* nonsense mutation (pDA7, Npp106Δ293-933) was able to partially complement the synthetic lethality, forming small colonies. A slightly longer protein (pDA4, Npp106Δ349-933) was able to complement SL64. This indicated that the region between amino acids 292 and 348 was required for complementation. When this region was deleted from the full-length protein (pDA9, Npp106pΔ265-343), it formed microcolonies. We then tested whether any sequences upstream of amino acid 265 were required. Whereas deletion of the first heptad repeat region (pDA10, Npp106pΔ28-64) complemented the synthetic lethality, deletion of the second and third heptad repeat regions (pDA8, Npp106pΔ74-143) could not.

We then examined the ability of these plasmids to complement SL64 in liquid cultures. None of the plasmids affected the growth rate of SL64 grown in the absence of thiamine (Fig. 1C). Additionally, they did not affect the growth of either the Δ*npp106* mutant or the *rae1-167* mutant grown at a permissive temperature (data not shown). Thus, expression of the deletion proteins did not interfere with growth in a *rae1*$^+$ background and is recessive to *npp106*$^+$ in a *rae1-167* background. Addition of thiamine to SL64 cells carrying the pDW232 vector resulted in cessation of growth at approximately 30 h.

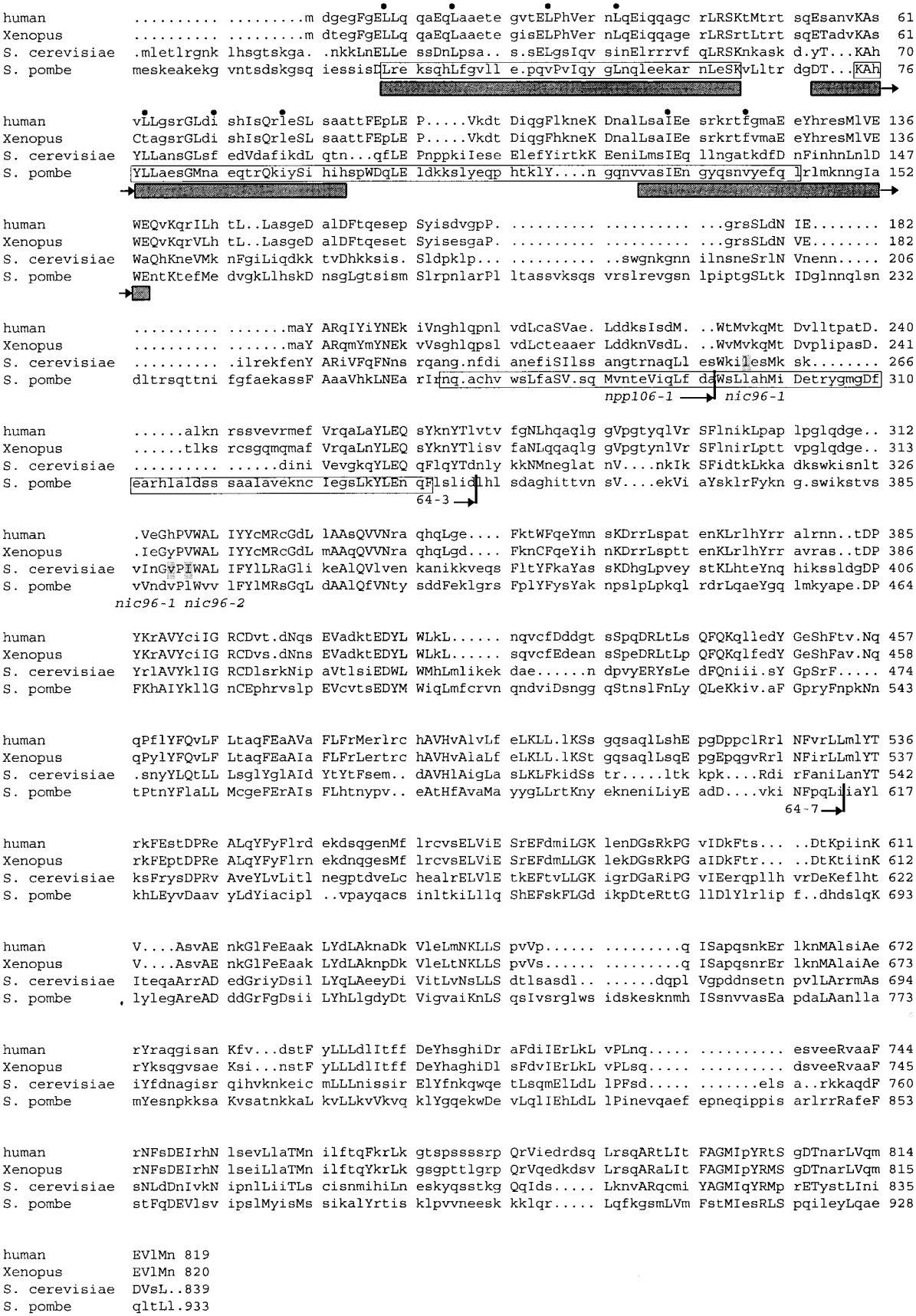

FIG. 2. Alignment of sequences of proteins with similarity to Npp106p: Nic96p (31) from *S. cerevisiae*, An4a (accession number, U63919) from *X. laevis*, and a predicted protein from *H. sapiens* (D42085). The alignment was generated by using the PILEUP program of the Wisconsin Package. A plurality of three identities is shown by capital letters. Heptad repeat regions that were identified by Grandi et al. (33) are identified by shaded boxes beneath the sequence. Conserved hydrophobic residues within the heptad repeats are identified by dots above the residues. Sequences deleted from Npp106p are identified by boxes around the sequences. The locations of truncations are indicated by vertical lines. Mutations of *NIC96* (77) are highlighted and denoted below the sequence.

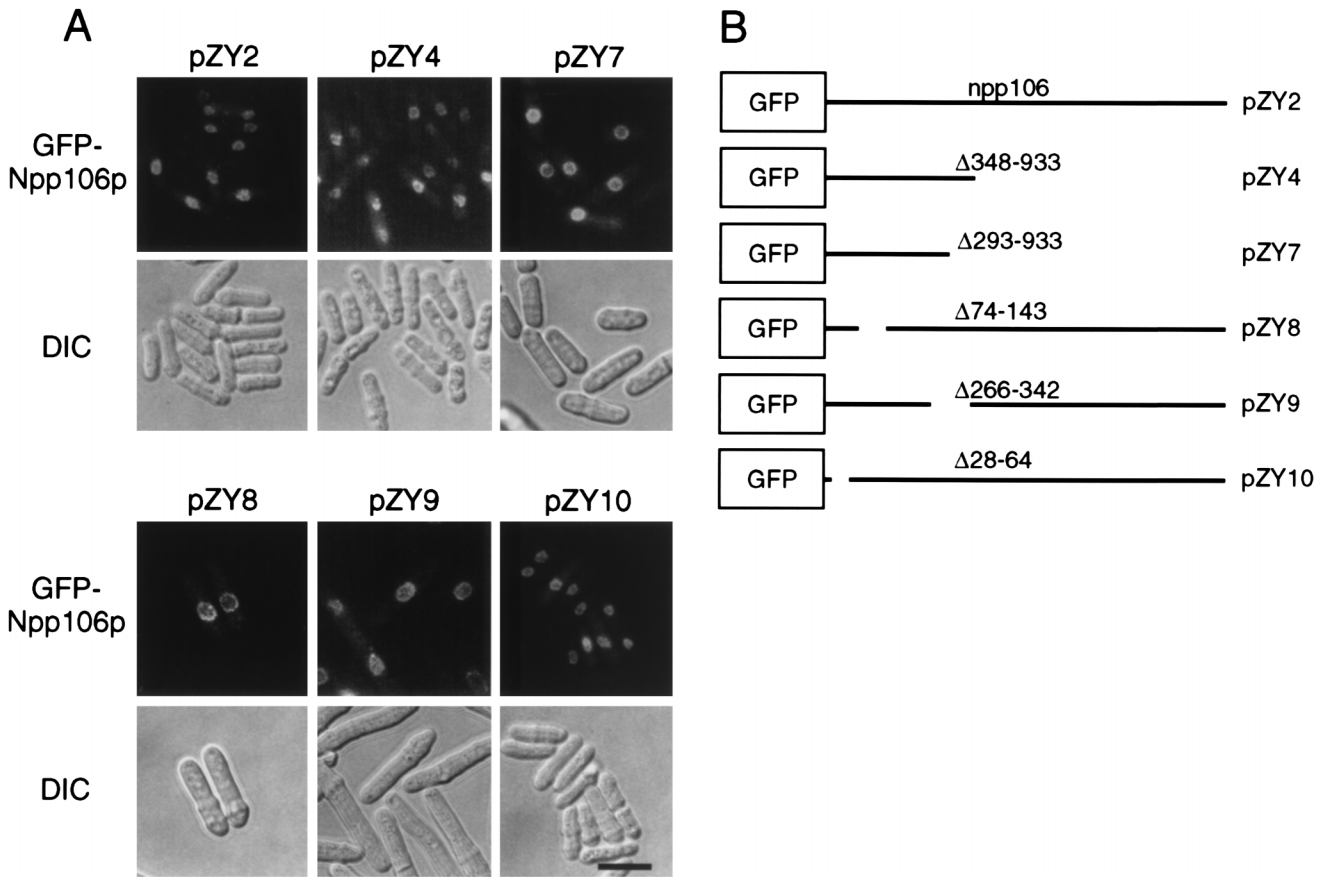

FIG. 3. Localization of Npp106p to the nuclear periphery. (A) GFP was fused to full-length Npp106p as well as to proteins with deletions to the amino termini and expressed from the thiamine-repressible *nmt* promoter on pZA69U in Δ*npp106* (JBP172) cells. Cells were grown in appropriately supplemented EMM medium in the presence of thiamine to mid-log phase, and the localization of the GFP fusion proteins were examined in live cells (upper panels). Coincident Nomarski (differential interference contrast [DIC]) images are shown in the lower panels. Bar, 10 μm. (B) Maps of the GFP fusion proteins.

Growth of SL64 bearing the deletion plasmids reflected their growth on plates: pDA8 was unable to sustain growth, pDA7 and pDA9 allowed slow growth, and pDA4 and pDA10 allowed good growth (Fig. 1C). These results define a region in Npp106p from amino acid 74 to 348 that is required for growth in a *rae1-167* background.

Since the Δ*npp106* mutation was synthetically lethal with *rae1-167*, one possibility for the inability of the deletions to complement SL64 is that they reduced the steady-state levels of Npp106p. Thus, we determined the relative steady-state levels of Npp106p expressed from pDA2, pDA8, pDA9, and pDA10 in SL64 grown under permissive conditions. Npp106p was detected by using a polyclonal antibody raised against an N-terminal peptide (residues 1 to 20) (Fig. 1D). This antibody detected a 106-kDa band in SL64 cells carrying pDA2 but not in SL64 cells. Whereas pDA9- and pDA10-bearing strains had a higher steady-state level of Npp106p than the pDA2-bearing strain, no Npp106p could be detected in the strain bearing pDA8. The nucleoporin-specific MAb 414 was used as a control for the amount of protein loaded onto the gels (Fig. 1D). The amount of Npp106p expressed from pDA4 was comparable to that expressed from pDA2, but the amount of Npp106p expressed from pDA7 could not be determined because of its comigration with a preimmune band (data not shown). Similar results were obtained when these proteins were expressed in the Δ*npp106* mutant (data not shown). While the inability of pDA8 to complement SL64 could be due to low steady-state levels of Npp106p(Δ74-143), the reduced complementation of pDA9 was not due to reduced Npp106p(Δ265-343) levels.

Npp106p localizes to the nuclear periphery. To determine the subcellular localization of Npp106p, we fused GFP to Npp106p at the amino terminus and expressed the fusion from the wild-type thiamine-repressible *nmt* promoter on pREP3 (Fig. 3B). This fusion is functional, since it can complement SL64 even when expression is fully repressed in the presence of thiamine at 27°C. To eliminate localization to the NPC through interaction with endogenous Npp106p (see below), the localization was examined in Δ*npp106* cells. These cells were then grown under repressing conditions in the presence of thiamine. Examination of live cells revealed that the Npp106p-GFP fusion was concentrated at the nuclear periphery with a punctate appearance (Fig. 3A). This distribution is similar to that observed for staining by MAb 414, which recognizes nucleoporins (see Fig. 6), and for the epitope-tagged AU1-Rae1p (75). These results suggest that like Nic96p in *S. cerevisiae*, Npp106p may be a component of the NPC.

We then constructed GFP fusions to the deletion proteins (Fig. 3B) to determine whether these deletions affected the ability of Npp106p to localize to the NPC. The cells were grown under repressing conditions, and the distributions of the fusions were examined. All of the GFP-tagged deletion proteins localized to the nuclear periphery, suggesting that they assembled into the NPC (Fig. 3A). Thus, amino acids 1 to 292

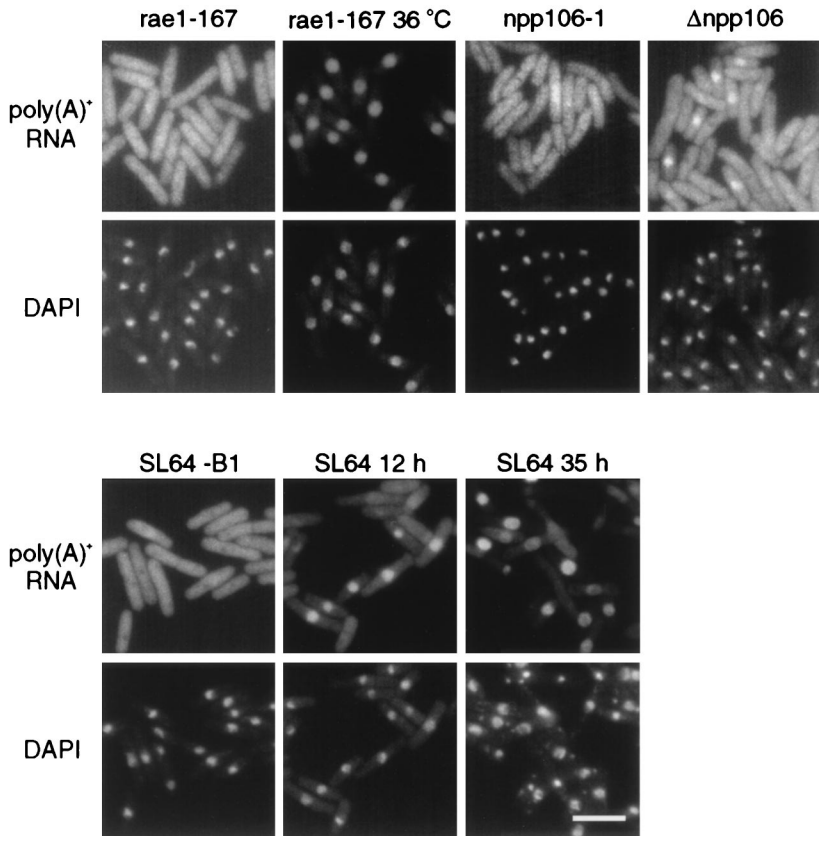

FIG. 4. Poly(A)$^+$ localization in *rae1-167* (JBP167), *npp106-1* (JBP171), Δ*npp106* (JBP172), and SL64 cells. Mutants were grown at 27°C to mid-log phase and stained for poly(A)$^+$ RNA. Coincident DAPI (4′,6-diamidino-2-phenylindole) staining is shown below each panel. The *rae1-167* mutant was also grown at 36°C for 1 h as indicated. SL64 was grown in the absence of thiamine (−B1) and in the presence of thiamine for the indicated amount of time. Bar, 10 μm.

are sufficient to target Npp106p to the NPC, and the deletions did not remove regions necessary for localization to the NPC.

The synthetic lethality confers an mRNA export defect. We then determined the influence of *npp106-1* on the export of mRNA in *rae1*$^+$ and *rae1-167* backgrounds (Fig. 4). Neither the *rae1-167* nor the *npp106-1* mutant had observable defects in mRNA export when grown at 27°C. When *rae1*$^+$ was expressed from the thiamine-repressible *nmt81* promoter in the *rae1-167 npp106-1* double mutant (SL64) in the absence of thiamine, there were no observable defects in mRNA export. However, after repression of *rae1*$^+$ for 12 h by growth of SL64 at 27°C in the presence of thiamine, 60% of the cells had extensive poly(A)$^+$ RNA accumulation in the nucleus, with lesser accumulation in the remainder of the cell. With longer repression of *rae1*$^+$ expression (35 h), the fraction of cells with defective mRNA export increased to more than 90%. Importantly, the Δ*npp106* mutant, which has no significant growth defects, has a detectable accumulation of mRNA in the nuclei of 20% of the cells. The normal poly(A)$^+$ RNA distribution in the Δ*npp106* mutant was restored by the plasmids that could complement SL64 (pDA2, pDA4, and pDA10 [Fig. 1]) but not by the plasmids that were unable to complement SL64 (pDA8 and pDA9) (data not shown). Taken together, our results suggest that *rae1* and *npp106* genetically interact to affect the export of poly(A)$^+$ RNA out of the nucleus.

No cytoplasmic mislocalization of a nuclear reporter protein in *rae1* and *npp106* mutants. Since Nic96p is required for import of a Matα2-LacZ reporter protein (33), we examined the localization of an SV40 NLS-GFP-LacZ reporter protein in the Δ*npp106*, *rae1-167*, and SL64 strains. In wild-type cells, essentially all the fusion protein was localized to the nucleus and no protein was detected in the cytoplasm (Fig. 5). As a control for the import of this reporter protein, we examined its localization in a nucleocytoplasmic trafficking mutant, the *ptr3-1* mutant, which has a temperature-sensitive E1 ubiquitin-conjugating enzyme and displays a nuclear protein import defect at restrictive temperature (9). The reporter protein was localized to the nucleus when *ptr3-1* cells were grown at the permissive temperature (25°C) and accumulated in the cytoplasm following growth for 3 h at the nonpermissive temperature (37°C). In Δ*npp106* cells, there was no detectable cytoplasmic accumulation of the reporter protein. Since there was little mRNA export defect in the Δ*npp106* cells, there was likely enough expression of reporter protein to detect accumulation in the cytoplasm. Therefore, there appears to be no detectable protein import defect of the reporter protein in Δ*npp106* cells.

Determination of whether a protein import defect exists in either the *rae1-167* or SL64 strain is more problematic. When *rae1-167* cells were grown at the restrictive temperature of 36°C for 4 h, no cytoplasmic accumulation was observed. However, under the respective restrictive conditions for these mutants, there might not be sufficient export of mRNA to synthesize enough reporter protein to detect its cytoplasmic accumulation. To attempt to circumvent this problem, *rae1-167* cells were grown at a semipermissive temperature of 31°C. This was done to allow sufficient expression of reporter protein under conditions of partial *rae1* function, where mRNA export

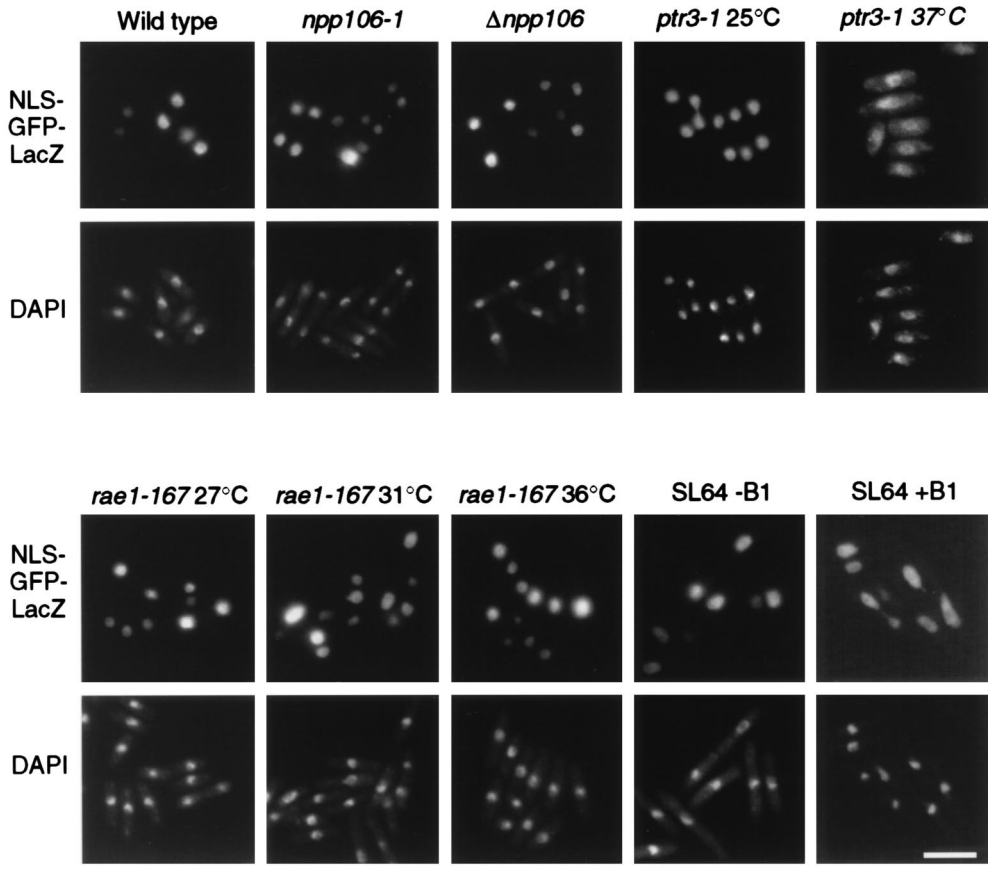

FIG. 5. Nuclear import of an SV40 NLS-GFP-LacZ reporter protein in wild type, *prt3-1* (9), Δ*npp106* (JBP172), *rae1-167* (JBP167), and SL64 cells. Plasmids expressing the SV40 NLS-GFP-LacZ reporter protein expressed from the thiamine-repressible *nmt* promoter were introduced into the indicated strains (the fusion was expressed from pREP41X for the *ptr3-1* strain and from pREP4 for the other strains). Wild-type, *npp106-1*, and Δ*npp106* cells were grown in EMM medium without thiamine at 27°C to mid-log phase, fixed, and examined for the localization of the reporter protein (upper panels). Coincident DAPI (4′,6-diamidino-2-phenylindole) staining is shown (lower panels). *ptr3-1* cells were grown to mid-log phase in EMM medium at 27°C and then shifted to the restrictive temperature of 37°C for 3 h. *rae1-167* cells were grown in EMM medium at the permissive temperature of 27°C, at the semipermissive temperature of 31°C for 6 hours, and at the nonpermissive temperature of 36°C for 4 hours. SL64 cells were grown at 27°C in the absence of thiamine (−B1) and in the presence of thiamine (+B1) for 24 h. Bar, 10 μm.

is partly defective (44). Examination of the cells grown under these conditions over a 24-h period (the 6-h time point is shown in Fig. 5) revealed that there was no detectable accumulation of reporter protein in the cytoplasm. Similarly, examination of the SL64 mutant from 12 to 40 h following repression of *rae1*$^+$ did not reveal any cytoplasmic accumulation of reporter protein at any time (the 40-h time point is shown in Fig. 5). While no protein import defect could be detected in the *rae1-167* and SL64 strains, it is still possible that there was a defect but that the synthesis of reporter protein was not sufficient to allow detectable cytoplasmic accumulation due to decreased mRNA export.

NPC distribution and nuclear envelope morphology are unaffected in *rae1* and *npp106* mutants. For *S. cerevisiae*, several mutants defective in nuclear export of poly(A)$^+$ RNA also have abnormalities in NPC distribution, with pores clustering to one side of the nucleus (summarized in reference 14). Specifically, the *gle2-1* mutant shows this abnormal distribution within 3.5 h after a shift to a restrictive temperature (57). However, incubation of the *rae1-1* mutant at a restrictive temperature for 1 h did not result in any abnormal distribution of the nuclear pores (75). Since the difference between these studies was the time of incubation at the restrictive temperature, we examined the nuclear pore distribution of *rae1-167* cell grown at a nonpermissive temperature up to 5 h (the 4-h time point is shown in Fig. 6). Longer times were not examined because the viability of the *rae1-167* mutant drops below 15% after a 5-h incubation at the restrictive temperature. Unlike the case for *gle2-1* cells, the NPC distribution was indistinguishable from that of wild-type cells and *rae1-167* cells grown at the nonpermissive temperature. Additionally, nuclear pore distribution was unaffected in Δ*npp106*, in *npp106-1*, and in SL64 cells when *rae1*$^+$ expression was repressed for 40 h (Fig. 6). The pore distribution was also examined at earlier times of *rae1*$^+$ repression, with no observable effect on NPC distribution (data not shown).

The *nic96-1* and *nic96-2* temperature-sensitive mutants have a three- to fourfold lowering of NPC density after growth at a restrictive temperature (77). The NPC densities in wild-type and Δ*npp106* cells were compared by examination of thin-section electron micrographs of 12 nuclei each, with the number of pores per length of nuclear envelope determined (Fig. 7). The NPC density was 1.19 ± 0.09 NPC/μm for wild-type cells and 1.20 ± 0.08 NPC/μm for Δ*npp106* cells. Thus, the loss of *npp106* function does not affect NPC density.

Electron microscopic examination of *gle2-1* mutant cells after incubation at the restrictive temperature revealed several morphological defects: clustered nuclear pores, herniated nuclear membranes, and intranuclear annulate lamella-like structures (57). However, examination of the nuclear envelope

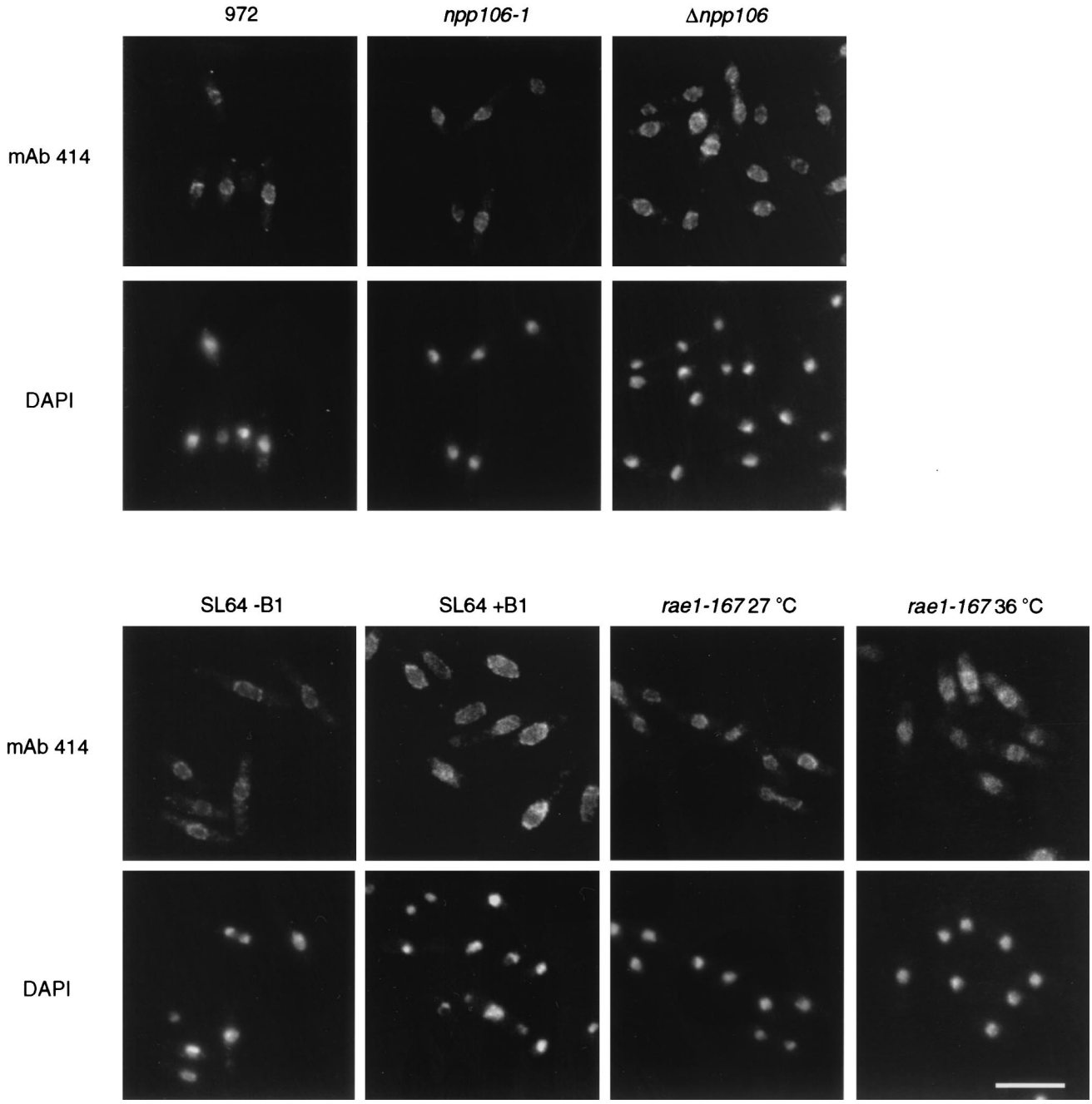

FIG. 6. Immunolocalization of nuclear pores in wild-type (972), *npp106-1* (JBP171), Δ*npp106* (JBP172), *rae1-167* (JBP167), and SL64 cells. In the upper panels, nuclear pores were stained with MAb 414 (8), and coincident DAPI (4′,6-diamidino-2-phenylindole) staining is shown in the lower panels. All cells were grown to mid-log phase in appropriately supplemented EMM medium at 27°C. The *rae1-167* mutant was also incubated at the restrictive temperature of 36°C for 4 h, and SL64 was grown in the presence of thiamine (+B1) for 40 h. Bar, 10 μm.

structure of *rae1-167* cells grown at permissive and restrictive temperatures showed no herniated nuclear membranes or intranuclear annulate lamella-like structures (data not shown). Additionally, SL64 cells grown with and without $rae1^+$ expression and Δ*npp106* cells also showed no nuclear envelope perturbation (data not shown). The nuclear pore distribution and nuclear envelope perturbations in *gle2* mutants have been suggested to be pleiotropic effects, since these defects were seen well after the time that the mRNA export defect could be detected (57). From the studies described above, we conclude that there are no gross changes in either NPC distribution or nuclear envelope structure and integrity in either the *rae1-167*, Δ*npp106*, or SL64 mutant.

Self-interaction of Npp106p. We next wanted to determine whether Rae1p could physically interact with Npp106p either directly or indirectly as part of the same subcomplex. However, coimmunoprecipitation experiments using crude cell extracts could not be performed, since there were not sufficient detectable amounts of soluble Npp106p. We then performed a two-hybrid experiment. Full-length Rae1p was fused to the activa-

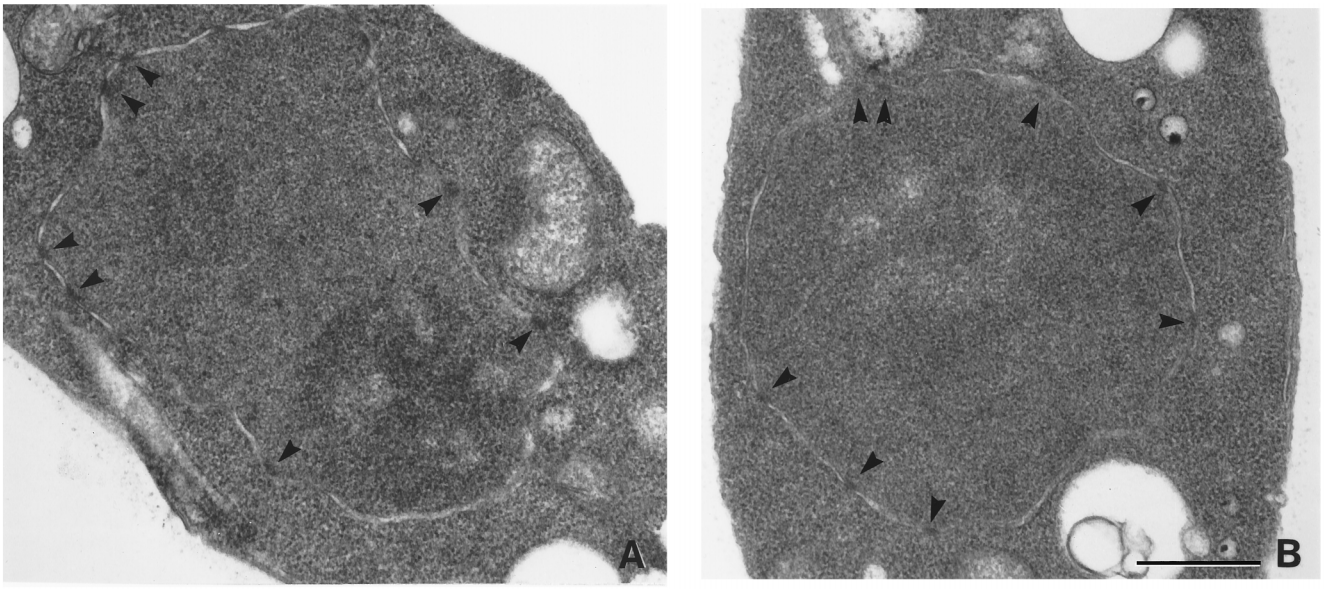

FIG. 7. Thin-section electron micrographs of wild-type (972) (A) and Δ*npp106* (JBP172) (B) cells. Nuclear pores are indicated by arrowheads. Bar, 0.5 μm.

tion domain of Gal4p, and full-length Npp106p was fused to the DNA binding domain of Gal4p. The amount of β-galactosidase synthesis was determined by using a filter assay (Fig. 8). No direct interaction between Rae1p and Npp106 was detected by using the two-hybrid system. This does not rule out the possibility that Rae1p and Npp106p do not directly interact or are constituents of the same functional subcomplex or that an additional protein is required to stabilize a Rae1p-Npp106p interaction. Interestingly, when full-length Npp106p was fused to both the Gal4p activation and binding domains, there was significant transcription of *lacZ* that was comparable to the activation resulting from an interaction between p53(72-390) and SV40 T antigen(84-708) (41). The interaction of Npp106p with itself appears to be specific, since no significant transcription of *lacZ* was observed in strains bearing the Npp106p-$Gal4_{AD}$ fusion and either the Rae1p-$Gal4_{AD}$ or p53(72-390)-$Gal4_{AD}$ fusion as well as in a strain carrying the Npp106p-$Gal4_{BD}$ and SV40 T antigen(84-708)-$Gal4_{AD}$ fusions. This interaction was confirmed by the in vitro binding of ^{35}S-labeled Npp106p to a GST-Npp106p fusion (Fig. 8B). Therefore, there appears to be a self-association of Npp106p.

DISCUSSION

In fission yeast, *rae1* is an essential gene required for export of poly(A)$^+$ RNA from the nucleus (11). In *S. cerevisiae*, a temperature-sensitive mutant of the *rae1* homolog, *gle2-1*, rapidly accumulates mRNA in the nucleus when shifted to restrictive temperature. Epitope-tagged Rae1p in *S. pombe* and Gle2p in *S. cerevisiae* localize to the nuclear periphery with a punctate appearance (57, 75), and Gle2p has been shown to copurify with a highly enriched NPC fraction (57). There is also a genetic interaction between *GLE2* and a GLFG repeat nucleoporin gene, *NUP100* (57). While no direct physical interaction has yet been detected between Gle2p and Nup100p, as well as the related GLFG repeat proteins Nup116 and Nup145p, a potential physical interaction has been detected between Gle2p and a nucleoporin-like protein, Nup42p/Rip1p, by using a two-hybrid system (57). Also, Gle2p has been isolated in a complex that includes Nup116p (40). Here we report a genetic interaction between *rae1* and *npp106*, whose predicted protein product has significant similarity to the nonrepeat nucleoporin of *S. cerevisiae*, Nic96p (31). The loss of *npp106* function results in synthetic lethality in a conditional *rae1-167* mutant. One major consequence of this synthetic lethality is the inability to export poly(A)$^+$ RNA from the nucleus. This genetic interaction uncovers a direct or indirect involvement of Npp106p in mRNA export.

In *S. cerevisiae*, Nic96p copurifies with a highly enriched NPC (3). It has been shown to genetically and physically interact with other nucleoporins, Nup188p and the Nsp1p-up49p-Nup57p complex (33, 60, 77). Npp106p localizes to the nuclear periphery in a punctate pattern (Fig. 3) that is similar to the pattern for other nuclear pore proteins. Since Nic96p is

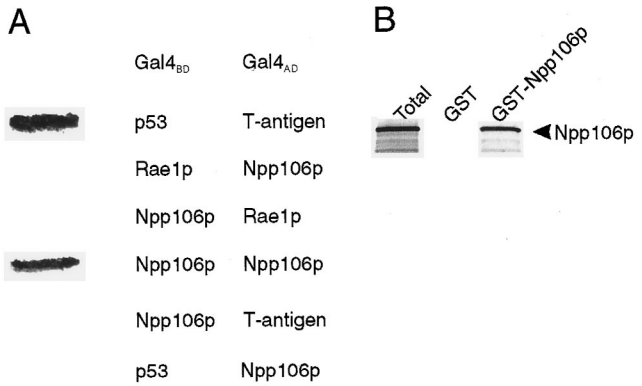

FIG. 8. Interaction of Npp106p with itself and Rae1p. (A) Two-hybrid analysis. Plasmids expressing full-length fusions of Rae1p and Npp106p to both the Gal4p activation domain ($Gal4_{AD}$) and the Gal4p DNA binding domain ($Gal4_{BD}$) as well as control plasmids expressing p53(72-390)-$Gal4_{AD}$ and SV40 T antigen(84-708)-$Gal4_{BD}$ fusions were introduced into the *S. cerevisiae* Y190 reporter strain (35) as indicated. LacZ production was determined by filter assay. (B) In vitro binding experiments. Npp106p and Rae1p were synthesized in vitro and labeled with [^{35}S]methionine in rabbit reticulocyte lysates. These extracts were incubated with GST and GST-Npp106p beads and washed, and the bound protein was eluted and run on an SDS-polyacrylamide gel.

a major constituent of the nuclear pore (3), it is likely that Npp106p is also a nucleoporin.

Despite the strong sequence similarity between Npp106p and Nic96p, there appear to be differences in their functions in *S. pombe* and in *S. cerevisiae*. Whereas *npp106* is not essential, *NIC96* is (31). Moreover, depletion of Nic96p leads to a defect in nuclear import of a Matα2-LacZ reporter protein (33), but the Δ*npp106* mutant showed no defect in the import of an SV40 NLS-GFP-LacZ reporter protein (Fig. 5). It remains to be determined whether the lack of *npp106* function affects the import of proteins bearing different NLS sequences. Finally, while depletion of Nic96p does not impair export of poly(A)$^+$ RNA, the Δ*npp106* mutant has a slight mRNA export defect (Fig. 4), but it is not severe enough to affect growth. The molecular basis for this apparent plasticity in function is not known, and its elucidation will require further knowledge of the genetic and physical interactions of Npp106p and Nic96p.

There are several possible reasons for this apparent plasticity. Npp106p may be functionally redundant in *S. pombe*, where another Npp106p-like protein in the NPC may be capable of substituting for Npp106p. While in *S. cerevisiae* there are no other Nic96p-like proteins that can substitute for the essential *NIC96* function, this cannot be ruled out for *S. pombe*. Similarly, there may be redundant nucleocytoplasmic transport pathways that are independent of Npp106p and Nic96p. The abilities of these pathways to substitute in the absence of the Npp106p- and Nic96p-dependent pathways may differ in these organisms. Another possibility is derived from the proposal that Nic96p links subcomplexes of the transport machinery to the structural framework of the NPC (60, 77). These subcomplexes may be functionally distinct: one for protein import and another for mRNA export. The function of these subcomplexes could be differentially affected by the strength of their interactions with other nucleoporins in the absence of Nic96p in *S. cerevisiae* and Npp106p in *S. pombe*. For example, in *S. pombe* the protein import subcomplex could be functionally stable in the absence of Npp106p through interactions with other nucleoporins, whereas the mRNA export subcomplex could be slightly unstable. Conversely, in *S. cerevisiae*, a protein import subcomplex (e.g., Nsp1p-Nup49p-Nup57p [see below]) could be destabilized by the absence of Nic96p, whereas an mRNA export subcomplex is stable.

Since Δ*npp106* is synthetically lethal with *rae1-167*, it appears that it is the loss of *npp106* function that gives rise to the synthetic lethality. Loss of *npp106* function also leads to a slight defect in mRNA export (Fig. 4). One possibility for the synthetic lethality is based upon the observation of reduced NPC density in the *nic96-1* and *nic96-2* temperature-sensitive mutants (77). The reduced NPC density could lower mRNA export capacity, which could be further impaired by the presence of mutant Rae1p. This does not appear to be the case, since NPC density was not reduced in a Δ*npp106* mutant (Fig. 7). Alternatively, removal of Npp106p from the NPC could affect the structure of the pore in such a way that mRNA export is impaired. Since protein import of an SV40 NLS-bearing reporter protein appears to be normal in Δ*npp106* and SL64 mutants (Fig. 5), perturbation of pore function appears to be limited. On the other hand, *rae1* and *npp106* could both affect the functioning of the mRNA export machinery. This may well be the case, since the loss of either *rae1* or *npp106* function results in a defect in mRNA export (Fig. 4). The genetic interaction between *rae1* and *npp106* could be indirect, where these mutations affect either two redundant pathways or two distinct steps in mRNA export. Alternatively, Rae1p and Npp106p could be members of the same subcomplex involved in mRNA export. Whereas the loss of Npp106p function would only partially inactivate this subcomplex, the presence of mutant Rae1p could further inhibit mRNA export.

Deletion analysis has identified a region in Npp106p between amino acids 74 and 348 that is required for complementation of SL64 (Fig. 1) and for restoration of normal mRNA export to the Δ*npp106* mutant (data not shown). Deletions in this region did not dominantly interfere with mRNA export function, since expression of the deletion proteins from multicopy plasmids (pDA8 and pDA9) did not affect growth of either the SL64, wild-type, or *rae1-167* strain (data not shown), and they did not exacerbate the mRNA export defect of the Δ*npp106* mutant (data not shown). The steady-state level of Npp106p(Δ74-143) expressed from pDA8 was below detectable levels, and this low level of expression is likely the reason for its inability to complement SL64. However, the steady-state level of Npp106p(Δ266-342) expressed from pDA9 was higher than that of the full-length Npp106p expressed from pDA2. Moreover this deletion did not affect the localization of GFP-Npp106p to the nuclear periphery. While localization to the nuclear periphery does not prove that this deletion protein properly assembles into the NPC, these results raise the possibility that this deletion removes a region of Npp106p that is required for proper functioning of the mRNA export machinery.

Nic96p contains three heptad repeats in its amino-terminal region, and the first of these repeats is required for interaction with the Nsp1p-Nup49p-Nup57p complex (33). Deletion of the first repeat confers temperature sensitivity and a defect in nuclear import of an NLS-bearing reporter protein at the restrictive temperature. Since *nsp1* and *nup49* temperature-sensitive mutants are defective in import of a reporter protein (16), it has been proposed that interaction of the Nsp1p-Nup49p-Nup57p complex with Nic96p is required for protein import (33). However, deletion of the analogous region in Npp106p (Δ28-64, pDA10) both can complement SL64 (Fig. 1) and does not show either an mRNA export defect or a growth defect in a Δ*npp106* strain (data not shown). It is unknown whether a complex analogous to the Nsp1p-Nup49p-Nup57p complex is present in *S. pombe* and, if present, whether this putative complex is functional in the absence of Npp106p.

Deletion of all three heptad repeats in Nic96p leads to a more severely altered phenotype, where the mutant grows slowly and nuclear import of a reporter protein is defective even at the permissive temperature. It is unknown whether the second or third heptad repeat of Nic96p is required for the interaction with Nsp1p-Nup49p-Nup57p complex, since deletion of only the first repeat is sufficient to prevent coimmune precipitation of this complex with Nic96p (33). Deletion of the region of Npp106p analogous to the second and third heptad repeats (Δ74-143, pDA8) was unable to complement SL64 and could not restore normal mRNA export to Δ*npp106*. This is likely a result of decreased steady-state levels of Npp106p. It is unknown whether removal of the second and third heptad repeats similarly reduces Nic96p levels in *S. cerevisiae.*

As mentioned above, it has been proposed that Nic96p links subcomplexes of the transport machinery to the structural framework of the NPC (60, 77). Moreover, the C-terminal region of Nic96p is required for interaction with a major structural nonrepeat nucleoporin, Nup188p. Though not essential, the C-terminal region of Nic96p may be important for proper interaction of the N-terminal region with the transport machinery, specifically the Nsp1p-Nup49p-Nup57p complex. Indeed, only the first 178 residues of Nic96p is sufficient for interaction with the Nsp1p-Nup49p-Nup57p complex. By analogy, the N-terminal region of Npp106p may also interact with the nucleocytoplasmic transport machinery. In support of this,

the first 348 amino acids of Npp106p was sufficient to complement SL64, restore normal mRNA export to the Δ*npp106* mutant, and be localized to the nuclear periphery. This localization may reflect its interaction with other components of the nuclear pore. We have not determined whether Npp106p interacts with a complex similar to the Nsp1p-Nup49p-Nup57p complex or with another subcomplex that potentially includes Rae1p. These studies have been technically difficult, since there was an insufficient amount of soluble Npp106p to perform coimmunoprecipitation experiments with crude cell extracts.

The synthetic lethality between *rae1* and *npp106* described here raises the possibility that, as has been suggested for protein import (60, 77), Npp106p/Nic96p may participate in linking the components of the mRNA export machinery to the structural components of the pore. In *S. cerevisiae*, there is a synthetic lethality between *gle2* and *nup100* mutations. Moreover, Gle2p can be isolated in a complex containing Nup116p (40). Nup100p and Nup116p, as well as Nup145p, contain GLFG repeats that have been proposed to be essential components of the mRNA export machinery. Indeed, a GLFG protein in *Xenopus* that is required for RNA export, Nup98, has been identified and may be the homolog of yeast Nup116p (65). Of particular interest in future studies will be the genetic and physical interaction of Rae1p/Gle2p, Npp106p/Nic96p, and the GLFG repeat proteins. Rae1p is highly conserved throughout evolution, and a human homolog is able to partially complement the *rae1-1* temperature sensitivity and mRNA export defect (10). Elucidation of Rae1p function in *S. pombe* will be important in understanding mRNA export function in general.

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