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Mutations affecting spindle pole body and mitotic exit network function are synthetically lethal with a deletion of the nucleoporin *NUP1* in *S. cerevisiae*

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Abstract

Nuclear pore complexes (NPCs) are embedded in the nuclear envelope of eukaryotic cells and function to regulate passage of macromolecules in and out of the nucleus. Nup1 is one of 30 nucleoporins comprising the NPC of the yeast *Saccharomyces cerevisiae* and is located on the nucleoplasmic face of the NPC where it plays a role in mRNA export and protein transport. In order to further characterize the function of Nup1 we used a genetic approach to identify mutations that are synthetically lethal in combination with a deletion of *NUP1* (*nup1Δ*). We have identified one such *nup1* lethal mutant (*nle6*) as a temperature sensitive allele of *nud1. NUD1* encodes a component of the yeast spindle pole body (SPB) and acts as scaffolding for the mitotic exit network (MEN). We observe that *nle6/ nud1* mutant cells have a normal distribution of NPCs within the nuclear envelope and exhibit normal rates of nuclear protein import at both the permissive and restrictive temperatures. *nup1Δ* also exhibits synthetic lethality with *bub2Δ* and *bfa1Δ*, both of which encode proteins that colocalize with Nud1 at spindle pole bodies and function in the mitotic exit network. However, we do not observe genetic interactions among *nle6/ nud1*, *bub2Δ* or *bfa1Δ* and mutations in the nucleoporin encoding genes *NUP60* or *NUP170*, nor is *nup1Δ* synthetically lethal with the absence of components downstream in the mitotic exit network, including Lte1, Swi5, and Dbf2. Our results suggest a novel functional connection between Nup1 and proteins comprising both the spindle pole body and early mitotic exit network.

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Keywords

Nucleoporin; Nuclear pore complex; Nuclear transport; Spindle pole body; Mitotic exit network; Cell cycle

Introduction

In eukaryotic cells, the nuclear envelope provides the double-membraned barrier separating the cytoplasm from the nucleus. In order to allow the passage of molecules between the cytoplasmic and nucleoplasmic compartments, the nuclear envelope is perforated with large, hetero-oligomeric protein structures termed nuclear pore complexes (NPCs). The NPCs are the sole mediators of nucleocytoplasmic transport across the nuclear envelope. While small molecules are able to passively diffuse through the channel formed within each NPC, the movement of molecules of more than about 50 kDa is regulated (reviewed in Pemberton and Paschal 2005). Each NPC is defined by a filamentous cytoplasmic region, a central transporter region, and a nuclear basket structure and is comprised of approximately 30 different NPC proteins (nucleoporins or Nups), which both generate the structure of the NPC and participate in the transport of substrates through the pore (Rout et al. 2000; Cronshaw et al. 2002; reviewed in Lim and Fahrenkrog 2006). Nup1 is a nucleoporin in *Saccharomyces cerevisiae* that is localized asymmetrically to the nucleoplasmic side of the NPC at the nuclear basket (Rout et al. 2000). Although *NUP1* is non-essential in most strain backgrounds, cells lacking *NUP1* (*nup1Δ)* exhibit temperature sensitive growth, as well as defects in mRNA export, nuclear protein import, and nuclear envelope structure (Bogerd et al. 1994; Schlaich and Hurt 1995).

Recently, there has been emerging evidence that the NPC is important in other processes in the cell beyond its function in nucleocytoplasmic transport. Various roles have been characterized for nucleoporins in gene regulation, apoptosis, the secretory pathway and cell cycle control (reviewed in Fahrenkrog et al. 2004). Since the NPC is the only conduit for transport of the many cargos that move between the nucleoplasm and cytoplasm, it is particularly well suited to act as a point of cell cycle control. Alteration of cargo protein localization by phosphorylation adjacent to nuclear localization signals has been a well characterized form of cell cycle regulation (Jans and Hubner 1996; Kaffman and O'Shea 1999). Cargo has also been shown to be compartmentalized by regulated transport. For example, the phosphatase Cdc14 is sequestered to the nucleolus to prevent export and mitotic exit (Visintin et al. 1999). Changes in the NPC have been shown to affect karyopherin binding to nucleoporins and alter transport of substrates temporally during the cell cycle (Makhnevych et al. 2003). Beyond their role in transport, NPCs also physically interact with two spindle assembly checkpoint proteins, Mad1 and Mad2 during the cell cycle (Iouk et al. 2002). Additionally, several connections have been made between the NPC and the spindle pole body (SPB), which is also embedded in the nuclear envelope. The SPB functions as the microtubule-organizing center in yeast and controls assembly and localization of microtubule-based cellular scaffolding as well as chromosome segregation via the mitotic spindle. The NPC and SPB share two components, Cdc31 and Ndc1 (Fischer et al. 2004; Chial et al. 1998). Ndc1 has been shown to play a role in the assembly and

insertion of both NPCs and the SPB into the nuclear envelope (Lau et al. 2004; Madrid et al. 2006).

Here we describe a novel connection between the nucleoporin Nup1 and components of the SPB and mitotic exit network (MEN), a cell cycle checkpoint whose protein components localize to the SPB. Previously, we performed a genetic screen to identify mutants in *S. cerevisiae* that exhibit synthetic lethality with *nup1Δ* and thus require *NUP1* for viability (Belanger et al. 1994). This screen led to the isolation of 17 *nup1Δ* lethal (*nle*) mutants, including alleles of genes encoding the nuclear transport protein Kap60 and the nucleoporins Nup170 and Nup82 (Belanger et al. 1994, 2004; Kenna et al. 1996). In this study, we identify *nle6* as an allele of *NUD1*, encoding a SPB protein and anchor for proteins in the MEN (Gruneberg et al. 2000). We also observe that deletions of SPB/MEN components Bfa1 and Bub2 are synthetically lethal with *nup1Δ*. The conditional *nle6/nud1* mutant does not significantly alter NPC localization or protein import kinetics, nor does it affect Bfa1 or Bub2 localization to the NPC. Our results implicate Nup1 and the NPC in a novel role for regulation of cell cycle progression.

Materials and methods

Yeast strains, media, and reagents

Yeast genetic manipulation, cell culture, and media preparation were performed as described (Guthrie and Fink 1991), as were all yeast transformations (Woods and Gietz 2001). Enzymes for molecular biology were purchased from New England Biolabs (Beverly, MA) and Sigma-Aldrich (St. Louis, MO) and were used as per manufacturer's instructions. Haploid yeast strains containing genomic deletions of *BFA1*, *BUB2*, *LTE1*, *SWI5*, *DBF2*, *NUP60* and *NUP170* were purchased from Open Biosystems (Huntsville, AL) and mated to produce the strains used in this study (Table 1). Haploid *nup1Δ* yeast strain KBY1447 was generated by transforming the *nup1Δ/NUP1* diploid strain from Open Biosystems with *CEN URA3 NUP1* (pLDB59), sporulating and dissecting the resulting diploids, and isolating KBY1158 (*nup1Δ* + pLDB59). Selection against pLDB59 was performed on plates containing 1 μg/ml 5-Xuoro-orotic acid (5FOA; Zymo Research, Orange CA) to generate KBY1447. Osmotic stress was assayed on solid YPD media containing 1 M sorbitol.

Cloning and rescue of *NUD1*

Yeast strain KBY10 containing *nle6* was isolated as described previously (Belanger et al. 1994). Strain LDY796 used for cloning *nle6* was generated by crossing KBY10 with L2612, sporulating, and isolating temperature sensitive spores lacking *nup1::LEU2*. Cloning by complementation of *nle6* temperature sensitivity was performed by transforming LDY796 with a yeast genomic library in pRS202 (Connelly and Hieter unpublished). Transformants were incubated 5 days at 24°C on SD-Ura media, then were replica plated to fresh SD-Ura and incubated at 37°C. Viable colonies were restreaked to 37°C. Plasmids were isolated from three transformants viable at 37°C using glass bead lysis and retransformed into LDY796 and KBY10 to confirm complementation of *nle6* temperature sensitivity and of *nup1Δ nle6* synthetic lethality. DNA sequencing of complementing plasmids confirmed the

presence of *NUD1* on all complementing plasmids. Complementation of *nle6*^{ts} by *NUD1* was confirmed using pSM783.

Genetic analysis of *nup* and MEN mutants

Tetrad analysis was performed by mating haploid strains (Table 1) containing a deletion of *NUP1*, *NUP60* or *NUP170* with various SPB or MEN-encoding gene deletion strains, all in the BY4741/4742 strain background (Open Biosystems, Huntsville, AL). These gene deletions were grown on media containing G418 (Gibco BRL, Gaithersburg, MD) and ClonNat (Werner Bioagents, Jena, Germany) to select for the associated drug resistance markers. Diploid colonies were suspended in 3 ml 0.3% KAc supplemented with required amino acids at 24°C to induce sporulation. The asci of the resultant tetrads were incubated in 2 mg/ml zymolyase and then 24 tetrads were dissected onto YPD plates and grown at 24°C. Haploid cells from each tetrad were examined for segregation of selective markers by using a 48-prong inoculator to transfer serially diluted cell suspensions onto selective plates. Resultant haploid strains were transferred to SD-Ura, -His, -Met, -Lys, YP-G418 and YP-ClonNat plates. In the case of *nup1Δ*, crosses were made using a parental strain containing a *CEN NUP1 URA3* plasmid (KBY1158) and the haploid progeny were transferred to 5FOA to select against the plasmid. All the plates were then incubated at 24°C, with the exception of YPD and 5FOA, one of each of which was also incubated at 30°C and 37°C.

Fluorescence microscopy

LDY1033 (wild-type) and KBY1294 (*nud1-G585E*) strains were transformed with plasmids according to Woods and Geitz (2001). pSW950 (Nic96-GFP) was cut with *Afl*II and integrated at *HIS3*. pSW956 (Nsp1-GFP) was cut with *Spe*I and integrated at *HIS3*. pRL282 (Bfa1-GFP) and pRL288 (Bub2-GFP) were cut with *Xcm*I and integrated at *URA3*. Cells were grown at 24°C and observed using direct fluorescence microscopy of cells in log phase and after 2–4 h shifts to 37°C. Images were captured using SPOT camera software (Diagnostic Instruments, Inc., Sterling Heights, MI) and final images were produced in Adobe Photoshop CS (Adobe Systems Inc., San Jose CA).

In order to examine protein import kinetics, strains W303 (wild type) and LDY796 (*nle6*), were transformed with plasmid pSV40-NLS-GFP (Shulga et al. 1996), grown in SD–Ura to A_{600} 0.05–0.2, treated with metabolic inhibitor and observed by direct fluorescence microscopy (Shulga et al. 1996) using a Nikon E600 epifluorescence microscope. Samples at 37°C were shifted to the non-permissive temperature for 2 h and treated as described (Belanger et al. 2004).

Results

In order to identify genes encoding proteins that functionally interact with Nup1, we carried out a large-scale screen for mutant alleles that are synthetically lethal with *nup1Δ*. In this screen, 17 *nup1Δ* lethal (*nle*) mutants were obtained, *nle1* through *nle17* (Belanger et al. 1994). Of these, five have been cloned and all have been alleles of genes encoding proteins known to be involved in nuclear transport: *nle1 = srp1/kap60*, the NLS-binding subunit of the karyopherin *a*/*b* heterodimer (Belanger et al. 1994); *nle2 = gle1*, an essential activator of

mRNA export (Murphy and Wente 1996; Alcazar-Roman et al. 2006; Kenna, Belanger, Davis unpublished); *nle3*/*nle17* = *nup170*, a nucleoporin important for NPC structure and assembly (Kenna et al. 1996); *nle4* = *nup82*, an essential nucleoporin at the cytoplasmic face of the NPC (Grandi et al. 1995; Hurwitz et al. 1998; Belanger et al. 2004); and *nle7* = *yrb1*, a yeast Ran-binding protein necessary for activating the Ran-GAP (Schlenstedt et al. 1995; Belanger and Davis unpublished).

In order to determine the gene mutated in the temperature sensitive *nle6* mutant, we transformed a yeast genomic library into a strain containing the *nle6* allele and identified a genomic region that complemented the temperature sensitivity of *nle6*. This region of genomic DNA contained three open reading frames, including one encoding the SPB protein Nud1. We obtained a centromeric plasmid containing *NUD1* and observed that the plasmid complemented both the *nle6* temperature sensitivity (Fig. 1) and the *nle6 nup1Δ* synthetic lethality (data not shown). DNA sequence analysis of the *nle6* allele revealed a single base substitution in *NUD1* in which adenine was replaced by guanine at nucleotide 1754. This substitution resulted in a missense mutation in which a glycine was replaced with a glutamic acid at amino acid 585, so we now refer to the *nle6* mutant allele as *nud1-G585E*. Interestingly, this exact *nud1* allele was also isolated in an independent mutagenic screen designed to identify yeast lysis mutants (Alexandar et al. 2004).

Since the *nud1-G585E* conditional allele was also isolated in a screen to identify cell lysis mutants (Alexandar et al. 2004), we sought to determine if disruptions of specific nucleoporins or MEN components also confer a cell lysis phenotype. Both the cell lysis phenotype and the temperature sensitivity of the *nud1-G585E* allele are suppressed by incubation on media providing osmotic support (Alexandar et al. 2004). In order to determine if the growth phenotype associated with a *NUP1* deletion could also be suppressed by osmotic support, we streaked wild type, *nud1-G585E*, and *nup1Δ* cells on plates containing 1 M sorbitol in YPD (Fig. 2) and incubated the plates at 24°, 30°, 32°, and 37° for 2–5 days. As expected, the *nud1-G585E* cells grew on 1 M sorbitol at all temperatures. However, the *nup1Δ* cells exhibited a lack of growth at elevated temperatures in either the presence or absence of sorbitol, and actually grew more slowly at 30°C on media containing sorbitol than on YPD (Fig. 2, upper right). An identical experiment was carried out using the conditional *nud1* alleles *nud1–2* and *nud1–44*, the *nup* mutants *nup60Δ* and *nup133Δ*, and MEN mutants *bfa1Δ*, *bub2Δ*, and *dbf2Δ*. Again, the conditionality of the *nud1* mutants was suppressed by 1 M sorbitol, while the *nup* mutants exhibited slightly slower growth on YPD containing sorbitol (data not shown). Thus, unlike *nud1* mutants, growth phenotypes caused by the absence of the Nups tested cannot be suppressed by osmotic support. No difference in growth was observed in the presence or absence of sorbitol for the MEN mutants *bfa1Δ*, *bub2Δ*, or *dbf2Δ* (data not shown).

Since we isolated *nud1-G585E* through a synthetic lethal interaction with a component of the nuclear pore complex, we investigated whether *nud1-G585E* mutant cells exhibit alterations in NPC function. In order to test for changes in NPC distribution in *nud1-G585E*, we transformed plasmids expressing chimeric fusions of GFP with the nucleoporins Nic96 and Nsp1 into wild type and *nud1-G585E* cells. Cells expressing Nic96-GFP (Fig. 3a) and Nsp1-GFP (data not shown) were observed by fluorescence microscopy. Nic96-GFP and

Nsp1-GFP exhibited the same punctate nuclear envelope staining in *nud1-G585E* as in wild-type backgrounds grown at both 24°C and 37°C, indicating that NPC distribution throughout the nuclear envelope is not detectably altered in *nud1-G585E* mutant cells.

In order to determine if a loss of Nud1 function affects nuclear protein import through the NPC, we observed the subcellular localization of several nuclear proteins in *nud1-G585E* mutant cells at permissive and restrictive temperatures. In an effort to identify whether a loss of Nud1 function affected the transport of specific karyopherins/ importins, we performed steady-state fluorescence microscopy on *nud1-G585E* mutant cells expressing a classical NLS (cNLS) imported by the Kap95/Kap60 karyopherin heterodimer (Enenkel et al. 1995), the NLS of Nab2 imported by the karyopherin Kap104 (Siomi et al. 1998; Lee and Aitchison 1999), and the Pho4-NLS imported by Kap121 (Kaffman et al. 1998). The intracellular localization of Nab2-GFP, Pho4-GFP, and cNLS-GFP fusions was predominantly nuclear in wild type and *nud1-G585E* cells at both 24°C and 37°C (Fig. 3b and data not shown), indicating that Kap60/95, Kap104, and Kap121-mediated protein import is retained in the absence of Nud1 function.

While steady-state localization of these reporters indicated that each could be imported into the nucleus in cells containing the *nud1* mutation, this experiment did not address whether the kinetics of import are altered in the absence of Nud1 activity. We next examined whether the rate of import of a nuclear protein containing a classical nuclear localization signal (cNLS) is altered in a *nud1-G585E* mutant. To this end, we performed a kinetic assay of nuclear transport (Shulga et al. 1996) by transforming wild-type and *nud1-G585E* cells with a plasmid expressing a cNLS–GFP fusion, equilibrating the cNLS–GFP reporter protein across the nuclear envelope using metabolic inhibitors, then rinsing away the inhibitors to allow nuclear import to occur and cNLS–GFP to re-accumulate in the nucleus. The relative rate of nuclear protein import was determined by calculating the percentage of cells exhibiting a predominantly nuclear accumulation of GFP at 2 min time intervals. Under all conditions tested, approximately 50% of the cells had nuclear fluorescence within 5 min after release from metabolic inhibition (Fig. 3c), indicating that cells containing *nud1-G585E* import the cNLS–GFP into the nucleus with kinetics similar to wild-type cells at 25° and after a 2 h shift to 37°C. These data suggest that the *nud1-G585E* mutation of *NUD1* does not detectably alter the rate of cNLS-mediated protein import through the NPC.

Nud1 is an important structural component of the outer plaque of the SPB, located on the cytoplasmic face of the nuclear envelope (Elliott et al. 1999). Since Nud1 also acts as scaffolding to anchor components of the MEN at the SPB, specifically the Bub2/Bfa1 complex (Gruneberg et al. 2000), we tested whether *bub2Δ* and *bfa1Δ* also exhibit synthetic lethality with *nup1Δ*. Haploid cells containing *bub2* or *bfa1* deletion alleles were mated with *nup1Δ* cells and the resulting diploid strains sporulated to generate tetrads containing haploid meiotic progeny. Synthetic lethality was assessed based on the growth of double mutant haploid strains identified by tetrad analysis. Cells containing single *bfa1Δ*, *bub2Δ*, or *nup1Δ* mutations are viable, while those deleted for both *nup1* and *bfa1* or *nup1* and *bub2* are inviable (Fig. 4). This synthetic growth defect provides further evidence for a functional interaction between Nup1 and components of the SPB and/or MEN.

In order to further investigate the relationship between Nup1 and MEN function, we crossed *nup1A* cells with mutants containing deletions of genes encoding the non-essential MEN proteins Lte1, Swi5, and Dbf2. Each of these proteins functions downstream of Bfa1 and Bub2 in the mitotic exit network (Cid et al. 2002). Tetrad analysis revealed that *nup1A* does not exhibit synthetic lethality with *lte1Δ*, *swi5Δ*, or *dbf2Δ* alleles (Table 2), suggesting that the functional interaction between Nup1 and the MEN may be specific to those components of the MEN that function early in the signaling cascade, especially those factors that negatively regulate the progression to exit from mitosis.

In order to determine if the genetic interactions we observed between NPC and SPB/MEN components were specific to *nup1Δ* or were a general phenotype of altered NPC structure or function, we repeated the genetic analyses described above using the nucleoporin mutants *nup60Δ* and *nup170Δ.* Nup60 is another FG nucleoporin that, like Nup1, is associated asymmetrically with the nuclear face of the NPC (Rout et al. 2000). Additionally, *nup60Δ* is synthetically lethal with *nup1Δ* (Fischer et al. 2002). Nup170 is localized symmetrically in the NPC, but *nup170* deletions exhibit chromosome segregation defects and synthetic lethality with *nup1Δ* (Kerscher et al. 2001; Kenna et al. 1996). We generated haploid spores containing both a deletion of *nup60* or *nup170* and a mutation in *nud1*, *bub2*, *bfa1*, or other MEN components. Examination of these spores revealed that all combinations of *nup*/MEN mutations tested remain viable (Table 2). Thus, we observed that, unlike *nup1Δ*, the *nup60Δ* and *nup170Δ* alleles do not exhibit synthetic lethality with *nud1-G585E, bub2Δ* or *bfa1Δ*. We conclude that the genetic interactions we observe between *nup1Δ* and *nud1, bub2*, and *bfa1* are specific and not a general characteristic of NPC mutants.

Nup1 is a member of the FG-repeat containing family of proteins that physically associates with karyopherins in mediating cargo transport through the NPC. Nup1 contains a large central domain comprised almost entirely of ‘FXFG’ repeats, as well as a shorter C-terminal domain that includes several more degenerate ‘FG’ repeats (Davis and Fink 1990). The FXFG repeats of Nup1 associate with several karyopherins, including Kap95 (Rexach and Blobel 1995), and deletion of these repeats results in synthetic genetic interactions with other nucleoporin mutants (Strawn et al. 2004), but the FXFG domain is not essential for efficient cargo transport through the NPC (Pyhtila and Rexach 2003; Zeitler and Weis 2004). In order to investigate whether the synthetic lethality we observed between *nup1Δ* and early MEN mutants was the result of the absence of Nup1 FXFG repeats, we generated double mutant cells containing a deletion of the Nup1 FXFG domain (*nup1ΔFXFG*) in combination with *nud1-G585E, bub2Δ*, and *bfa1Δ* mutations and examined the resulting cells for growth phenotypes. All of the cells containing deletions of *nud1*, *bfa1*, or *bub2* in combination with *nup1ΔFXFG* are viable (Table 2), suggesting that MEN/ *nup1Δ* synthetic lethality is independent of the karyopherin-binding Nup1 FXFG repeats.

Since the Bub2/Bfa1 complex binds at Nud1 to act in the MEN (Gruneberg et al. 2000) and *bub2Δ* and *bfa1Δ* also exhibit synthetic lethality with *nup1Δ*, we investigated whether the loss of Nud1 function in *nud1-G585E* cells was interfering with binding of Bub2/Bfa1 at the SPB. In order to test this, we observed the subcellular localization of Bub2 and Bfa1 in cells containing the *nud1-G585E* mutation. Plasmids expressing Bub2-GFP and Bfa1-GFP were transformed into wild type and *nud1-G585E* cells and localization of the GFP fusions was

observed by direct fluorescence microscopy. Both Bub2-GFP and Bfa1-GFP exhibited discreet SPB localization in wild type and *nud1-G585E* cells cultured at 24°C and at 37°C, suggesting that the temperature sensitive phenotype of the mutant is not a result of altered Bub2/Bfa1 localization (Fig. 5a).

Given the genetic connections we observed between Nup1, Bfa1, and Bub2, we also examined whether mutations altering Nup1 function resulted in changes in Bfa1 or Bub2 localization. Interestingly, we were unable to successfully introduce either a Bfa1-GFP or Bub2-GFP containing plasmid into *nup1Δ* cells (data not shown). We were able to obtain expression of Bfa1-GFP and Bub2-GFP in *nup1ΔFXFG* cells and the intracellular distribution of both fusion proteins appeared identical to the localization observed in wild type cells (Fig. 5b).

Discussion

Cells lacking the nucleoporin Nup1 exhibit defects in cell structure and function, including nuclear transport of proteins and RNAs, nuclear envelope morphology, nuclear inheritance, and microtubule organization (Bogerd et al. 1994; Schlaich and Hurt 1995; Fischer et al. 2002). We have previously described a large-scale genetic screen used to identify mutations that are synthetically lethal with a deletion of *NUP1* (Belanger et al. 1994). Seventeen *nup1Δ* lethal (*nle*) mutants were isolated in this screen, representing 16 complementation groups. Six of the *nle*s are alleles of genes encoding proteins involved in nuclear transport and/or NPC function. In this work, we report the cloning of *nle6* and its identification as a temperature-sensitive allele of *NUD1*, an important component of the yeast spindle pole body and regulator of activation of the mitotic exit network. We also identify deletions of the SPB and MEN components Bfa1 and Bub2 as synthetically lethal with *nup1Δ*. Thus, *nle6/nud1-G585E*, *bfa1Δ*, and *bub2Δ* represent the first *nle*s without a previously identified role in nuclear transport or NPC function and provide a potential link between Nup1 and the activity of the SPB and/or MEN.

Recently, a number of significant connections have been made between the NPC and cellular processes other than nucleocytoplasmic transport (see Fahrenkrog et al. 2004 for review), including links to SPB and MEN function in yeast. Ndc1 is a transmembrane protein that localizes to both NPCs and SPBs and is important for assembly of both of these massive complexes spanning the nuclear envelope (Chial et al. 1998; Lau et al. 2004; Madrid et al. 2006). The yeast centrin Cdc31 is a SPB-associated protein that is important for SPB duplication (Baum et al. 1986; Spang et al. 1993) and has recently been shown to be an important component of the Sac3-Thp1 mRNA export complex that binds Nup1 and Nup60 at the nucleoplasmic face of NPCs and plays an important role in mRNA export (Fischer et al. 2002, 2004). Additionally, the Mlp2 protein is associated with the nucleoplasmic face of the NPC, but also binds the SPB components Spc29, Spc42, and Spc110 and is important for SPB structure and function (Niepel et al. 2005). Our data describing a synthetic interaction between *nup1Δ* and mutations in *NUD1*, *BFA1*, and *BUB2* provide additional genetic evidence for a link between Nup1 and SPB function. Interestingly, Nup1 is located exclusively on the nucleoplasmic face of the NPC, while Nud1, Bfa1, and Bub2 all associate

with the cytoplasmic plaque of the SPB, making a direct physical interaction between Nup1 and these SPB components unlikely.

NPC components are also important in cell cycle regulation. The NPC participates in cell cycle progression in part by functioning as a passageway for the nucleocytoplasmic relocalization of specific proteins important for cell cycle control, such as the Swi6 transcription factor and the Cdc14 phosphatase (Queralt and Igual 2003; Harreman et al. 2004; Carmo-Fonseca et al. 2000). The yeast NPC itself undergoes a structural reorganization during mitosis that allows for altered nucleocytoplasmic transport of some proteins involved in mitotic progression (Makhnevych et al. 2003). Thus, the NPC functions to regulate the cell cycle in part via regulation of the nucleocytoplasmic localization of specific proteins important for cell cycle progression. Several genetic interactions have been identified that link specific nucleoporins and nuclear transport factors to MEN function in the cell cycle. Alleles of *NUP170* and *CDC14* were both isolated in a screen designed to identify mutations that have increased sensitivity to Cln2 overexpression (Yuste-Rojas and Cross 2000), alleles encoding the karyopherins Kap60, Mtr10, and Kap104 were isolated as suppressors of *cdc15* mutants (Shou and Deshaies 2002; Asakawa and Toh-e 2002), and Kap104 appears to function to stimulate Cdc14 activity and thus exit from mitosis (Asakawa and Toh-e 2002). Thus, the synthetic interaction between *nup1Δ* and MEN components described here may be the result of changes in nucleocytoplasmic transport. The lack of a nucleoporin such as Nup1 may alter the import or export of a factor or factors necessary for cell cycle progression in the absence of early MEN components, resulting in an inability to progress through the cell cycle.

Nud1 is a structural protein located on the outer plaque of the SPB on the cytoplasmic side of the nuclear envelope and also acts as scaffolding for the MEN (Wigge et al. 1998; Adams and Kilmartin 1999). Bub2 and Bfa1 are bound to the SPB as a complex by Nud1, where they act as a GTPase activating protein (GAP) to inhibit Tem1 (Gruneberg et al. 2000; Geymonat et al. 2002). The daughter-cell-specific Lte1 acts as a guanine nucleotide exchange factor (GEF) to activate Tem1 and facilitate its sequestering of Cdc15 to the daughter SPB (Wang et al. 2000; Gruneberg et al. 2000; Pereira et al. 2002). Here, Cdc15 stimulates the kinase activity of the Dbf2–Mob1 complex, which is also found in high concentrations at the daughter SPB (Cid et al. 2002). Activation of the Dbf2– Mob1 complex allows for total release of the Cdc14 phosphatase from the nucleolus into the cytoplasm, where it functions to reverse Cdk-dependent phosphorylation, inhibit mitotic Cdks and allow mitotic exit (Pereira et al. 2002; Visintin et al. 1998).

The synthetic interactions observed for *nup1Δ* with *nud1-G585E, bfa1Δ*, and *bub2Δ* suggest a functional interaction between Nup1 and components of the SPB and early MEN. The genetic interactions between this particular nucleoporin and SPB/early MEN components appear to be quite specific, as other nucleoporins and SPB/MEN components tested do not exhibit synthetic lethality (Table 2). Since proteins of the MEN are mostly localized to the cytoplasm, there is a physical separation between these and the Nup1 localized at the nuclear basket. While a direct physical link between Nup1 and the MEN components is unlikely, the shuttling between the cytoplasm and nucleoplasm of specific factors required for mitotic exit could implicate these proteins in the same functional pathway. Cdc14, a critical component

of the MEN, is sequestered in the nucleolus during much of the cell cycle, but must be exported into the cytoplasm to function in mitotic exit and thus *nup1Δ* mutants could alter Cdc14-mediated cell cycle progression (Carmo-Fonseca et al. 2000; Trautmann and McCollum 2005). However, although Nup1 could potentially facilitate nuclear export of Cdc14, it appears Cdc14 is exported by Crm1, an exportin that has not been shown to interact with Nup1 (Bembenek et al. 2005).

The specificity of the synthetic interaction between *nup1Δ* and components that function early in the MEN pathway, but not late MEN components, might provide some insight into the functional basis for this genetic interaction. Nud1, Bfa1, and Bub2 all function as negative regulators of cell cycle progression, acting to retain Tem1 in its inactive, GDP-bound state. The loss of this negative regulation may lead to an increased likelihood of cells exiting mitosis prematurely under some conditions (Bosl and Li 2005). Mutations in *nup1* not only affect nucleocytoplasmic transport, but also result in altered microtubule organization and aberrant nuclear inheritance, leading to an increase in multinucleate and anucleate daughter cells (Bogerd et al. 1994). Failure to regulate exit from mitosis under such conditions may lead to an increase in inviable daughter progeny. In contrast, the late MEN mutants tested (*lte1Δ*, *dbf2Δ*, *swi5Δ*) all encode proteins that stimulate mitotic progression (Bosl and Li 2005). Reduced amounts of these factors due to gene deletion could potentially result in a delay in progression that would allow appropriate chromosome segregation in a fraction of *nup1Δ* mutant cells, resulting in apparent viability of the *nup1A*/late MEN double mutants.

The genetic interactions presented here suggest that Nup1 may play a novel role in connection with the SPB and MEN. General defects in nucleocytoplasmic transport in *nup1Δ* do not seem to account for the connection. It is possible that specific Nup1-mediated transport of MEN proteins results in the genetic interactions between *nup1Δ* and mutants of the SPB/MEN. Careful localization of Cdc14 throughout the cell cycle in *nup1Δ* and other nucleoporin mutants may be necessary to elucidate whether nuclear transport is important for Cdc14 and MEN function and whether specific Nups are essential for this transport. Alternatively, Nup1 may function in mitotic progression via the MEN independently of its role in nucleocytoplasmic transport.

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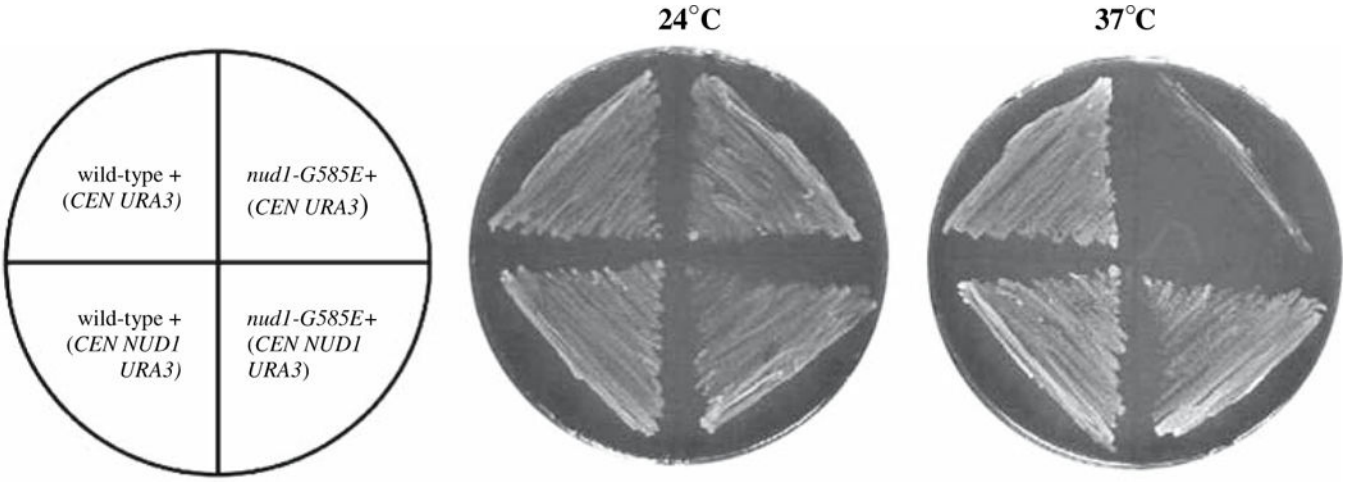

Fig. 1.

NUD1 complements the temperature sensitivity of *nud1-G585E.* Wild-type (LDY1033) and *nud1-G585E* (KBY1294) yeast were transformed with *CEN URA3* (pRS316) and *CEN NUD1 URA3* (pSM783) and streaked to four quadrants of –Ura plates (left). Cells were incubated at 24°C (middle) and 37°C (right) to visualize viability of the strains at these temperatures. *CEN NUD1 URA3* allows growth of *nud1-G585E* at 37°C

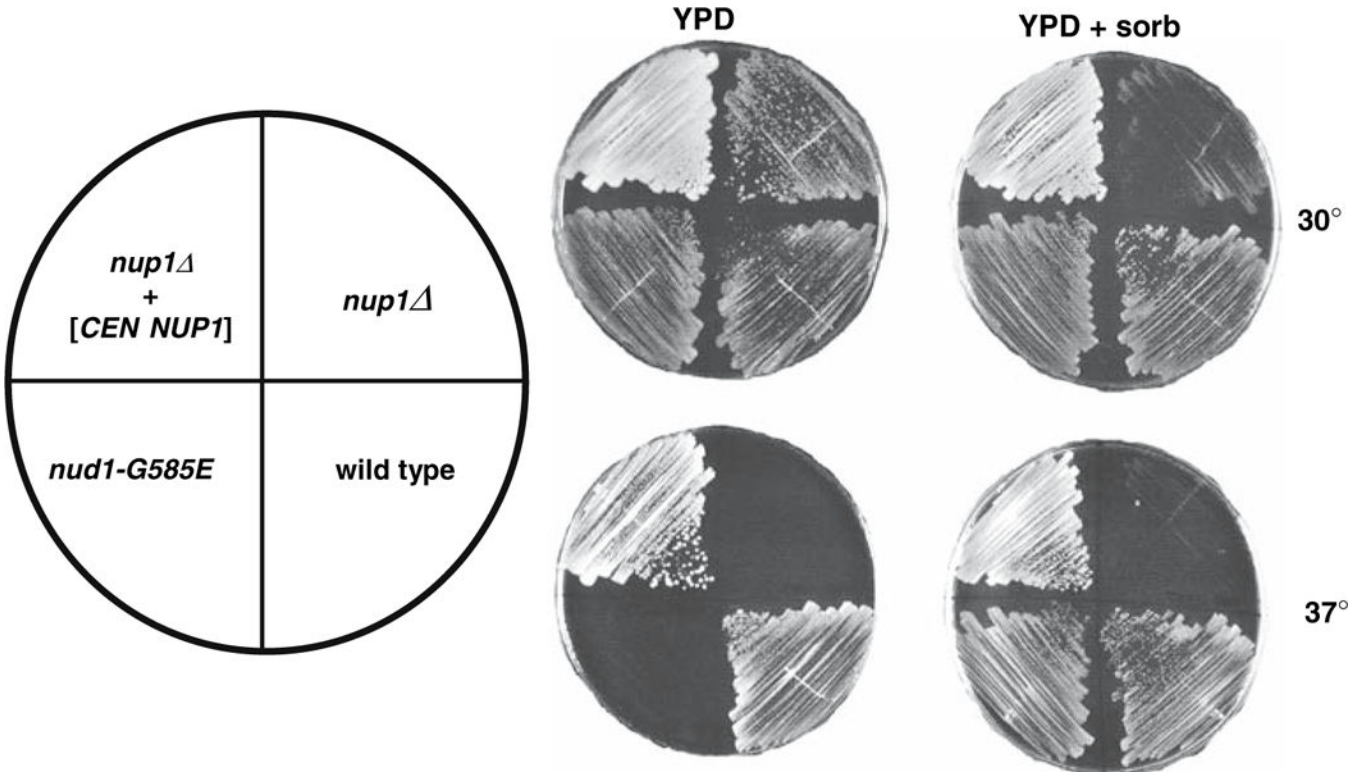

Fig. 2.

Osmotic support suppresses the temperature-sensitivity of *nud1-G585E* but not *nup1Δ*. Wild type haploid yeast (BY4742) and yeast containing *nup1Δ* (KBY1447), expressing *nup1Δ* covered by plasmid-borne *NUP1* (KBY1158), and containing *nud1-G585E* (KBY1294) were streaked to either YPD or YPD containing 1 M sorbitol (YPD + sorb) and incubated at 30 and 37°C. Cells were photographed 48–72 h after streaking

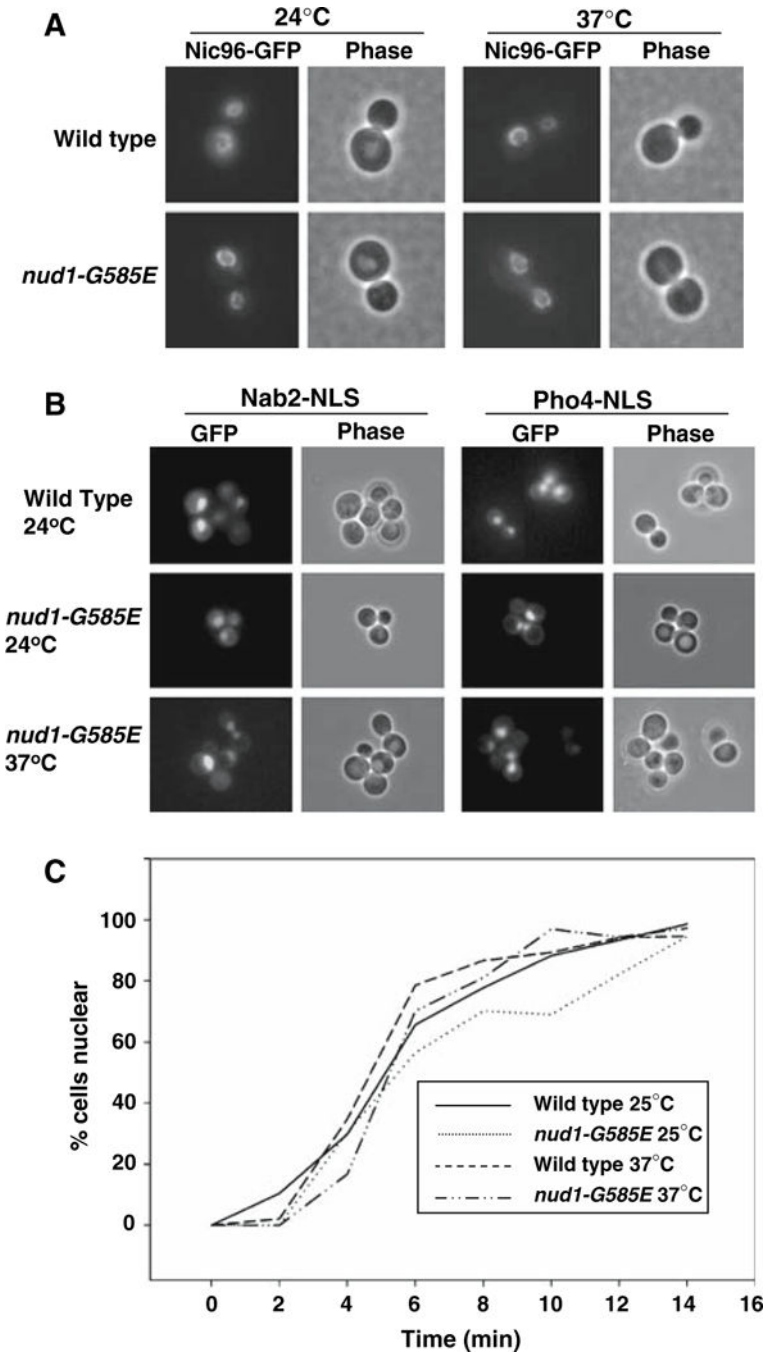

Fig. 3.

NPC distribution and nuclear protein import are not altered by the *nud1-G585E allele*. (**a**) Nic96-GFP localization was observed in log phase cultures that had been grown at 24°C (left) and then shifted to 37°C (right) for 4 h. Top panels show wild-type cells (W303) and bottom panels show *nud1-G585E* cells (KBY1294). Nic96-GFP localizes to the nuclear rim in a punctate manner in both wild-type background and *nud1-G585E.* (**b**) Wild type (BY4742) and *nud1-G585E* (KBY1294) cells were examined for subcellular localization of a GFP reporter fused to the Nab2 NLS (pNS167) and the Pho4 NLS (pEB0866) at 24°C and

after incubation at 37°C for 3 h. (**c**) Wild type (W303) and *nud1-G585E* (KBY1294) cells were assayed for import of a cNLS-GFP reporter after release from metabolic arrest (Shulga et al. 1996). The percentage of cells exhibiting nuclear fluorescence is plotted against the time elapsed after release from arrest

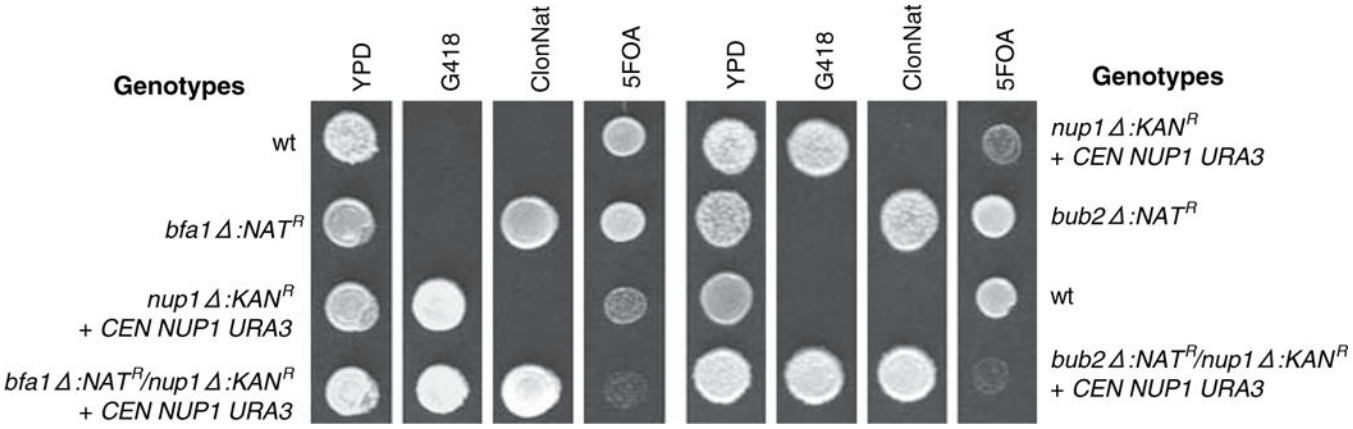

Fig. 4.

nup1Δ exhibits synthetic lethality with *bfa1Δ* and *bub2Δ*. Haploid *nup1Δ::KAN^R* (KBY1158) was mated with *bub2Δ::NAT^R* (KBY1225) and *bfa1Δ::NAT^R* (KBY1222) and the resulting diploid strains sporulated. Tetrad analysis was performed on 24 tetrads from both of these crosses. The four meiotic progeny of single representative tetratype tetrad containing spores of each of the four possible genotypes (wild-type, both single mutants and the double mutant) are shown for the *nup1Δ* × *bfa1Δ* cross (left) and the *nup1Δ* × *bub2Δ* cross (right). These genotypes were determined by plating spores to G418 and ClonNat as described in “Materials and Methods”. Spores were plated to 5FOA to induce loss of *CEN NUP1 URA3* so that the viability of the double mutant could be scored. Both *nup1Δ*/*bfa1Δ* and *nup1Δ*/*bub2Δ* double mutants fail to grow on 5FOA indicating a synthetic lethal interaction between these genes

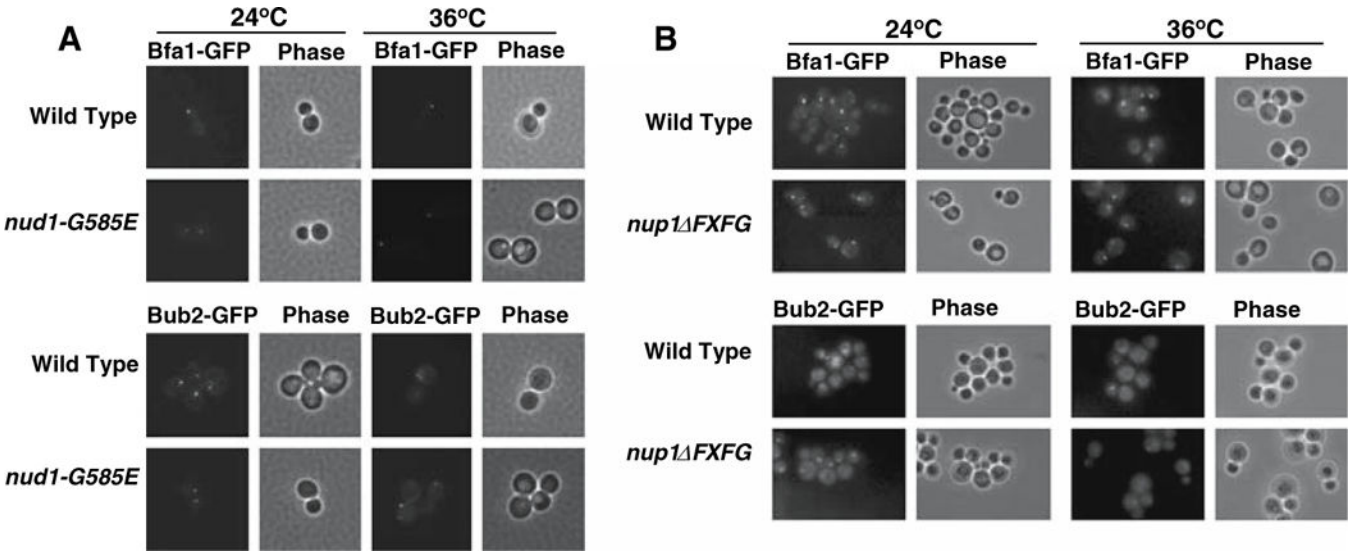

Fig. 5.

Bfa1 and Bub2 are localized to the SPB in *nud1* and *nup1* mutant cells. (**a**) Plasmids expressing Bfa1-GFP (pRL282) and Bub2-GFP (pRL288) were transformed into wild type (BY4742) and *nud1-G585E* (KBY1294) yeast and incubated on selective media. Bfa1-GFP (top) and Bub2-GFP (bottom) localization was observed in log phase cultures at 24°C (left) and after shift to 36°C for 4 h (right). (**b**) Bfa1-GFP and Bub2-GFP plasmids were transformed into wild type (BY4742) and *nup1AFXFG* (SWY2801) yeast and observed as described above. All cells retain Bfa1-GFP and Bub2-GFP localization to SPBs

Table 1

Yeast strains and plasmids used in this study

Yeast Strain	Relevant genotype	Source
W303	*MATa ade2 trp1 leu2 his3 ura3*	R. Rothstein
KBY52	*MATα nup1::LEU2 ade2 ade3 trp1 lys2 ura3* (*CEN URA3 ADE3 NUP1*) Strain background: W303	Belanger et al. (1994)
KBY124	*MATα nup1::LEU2 nud1-G585E^{ts} his3 ade2 ade3 trp1 ura3* (*CEN URA3 ADE3 NUP1*) Strain background: W303	Belanger et al. (1994)
KBY643	*MATα nup60::KAN^{R} his3 leu2 lys2 ura3*	Open Biosystems
KBY644	*MATα nup133::KAN^{R} his3 leu2 lys2 ura3*	Open Biosystems
KBY795	*MATα nup170::KAN^{R} his3 leu2 lys2 ura3*	Open Biosystems
KBY1113	*MATα bfa1::KAN^{R} his3 leu2 lys2 ura3*	Open Biosystems
KBY1115	*MATα bub2::KAN^{R} his3 leu2 lys2 ura3*	Open Biosystems
KBY1158	*MATa nup1::KAN^{R} his3 leu2 ura3 met15* (*CEN URA3 NUP1*)	This study
KBY1222	*MATα bfa1::NAT^{R} his3 leu2 lys2 ura3*	This study
KBY1225	*MATα bub2::NAT^{R} his3 leu2 lys2 ura3*	This study
KBY1294	*MATα nud1-G585E^{ts} his3 leu2 ura3 met15*	This study
KBY1345	*MATa lte1::KAN^{R} his3 leu2 met15 ura3*	Open Biosystems
KBY1346	*MATa dbf2::KAN^{R} his3 leu2 met15 ura3*	Open Biosystems
KBY1348	*MATa nup60::KAN^{R} his3 leu2 met15 ura3*	Open Biosystems
KBY1349	*MATa nup170::KAN^{R} his3 leu2 met15 ura3*	Open Biosystems
KBY1447	*MATa nup1::KAN^{R} his3 leu2 ura3 met15*	This study
BY4742	*MATa leu2 lys2 ura3 his3*	Open Biosystems
SWY2801	*MATα T7-loxP-nup1AFXFG trp1 ura3 leu2 his3*	Strawn et al. (2004)
KCY2-1	*MATa nud1–2::LEU2 nud1::KAN^{R} ura3 lys2 ade2 trp1 his3*	Gruneberg et al. (2000)
IAY520	*MATα nud1–44::TRP1 nud1::HIS5 ura3 ade3 leu2*	Adams and Kilmartin (1999)
Plasmid		
pRL282	*BFA1-GFP URA3*	Li (1999)
pRL288	*BUB2-GFP URA3*	Li (1999)
pRS314	*CEN TRP1*	Sikorski and Hieter (1989)
pRS316	*CEN URA3*	Sikorski and Hieter (1989)
pSM783	*CEN NUD1 URA3*	Gruneberg et al. (2000)

Yeast Strain	Relevant genotype	Source
pSW950	*NIC96-GFP HIS3*	Bucci and Wente (1998)
pUG115	*CEN NUD1 TRP1*	Gruneberg et al. (2000)
pLDB59	*CEN NUP1 URA3*	Bogerd et al. (1994)
pEB0866	*CEN URA3 PHO4-NLS-GFP*	Kaffman et al. (1998)
pNS167	*CEN URA3 GFP-Nab2(NAB35)*	Lee and Aitchison (1999)
pGAD-GFP	*CEN URA3 cNLS-GFP*	Shulga et al. (1996)

All yeast strains S288C unless otherwise noted

Table 2

Synthetic interactions between genes encoding nucleoporins and genes encoding MEN proteins

	wt	***nup1Δ***	***nup1ΔFXFG***	***nup60Δ***	***nup170Δ***
wt	+	+	+	+	+
nle6 (nud1-G585E)	+	SL	+	+	+
bfa1Δ	+	SL	+	+	+
bub2Δ	+	SL	+	+	+
lte1Δ	+	+	ND	+	+
swi5Δ	+	+	ND	ND	+
dbf2Δ	+	+	ND	ND	ND

SL: synthetic lethal interaction. +: no synthetic interaction was observed. ND: cross not performed