Nuclear Pore Complex Function in *Saccharomyces cerevisiae* Is Influenced by Glycosylation of the Transmembrane Nucleoporin Pom152p

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ABSTRACT

The regulated transport of proteins across the nuclear envelope occurs through nuclear pore complexes (NPCs), which are composed of >30 different protein subunits termed nucleoporins. While some nucleoporins are glycosylated, little about the role of glycosylation in NPC activity is understood. We have identified loss-of-function alleles of *ALG12*, encoding a mannosyltransferase, as suppressors of a temperature-sensitive mutation in the gene encoding the FXFG-nucleoporin *NUP1*. We observe that *nup1Δ* cells import nucleophilic proteins more efficiently when *ALG12* is absent, suggesting that glycosylation may influence nuclear transport. Conditional *nup1* and *nup82* mutations are partially suppressed by the glycosylation inhibitor tunicamycin, while *nic96* and *nup116* alleles are hypersensitive to tunicamycin treatment, further implicating glycosylation in NPC function. Because Pom152p is a glycosylated, transmembrane nucleoporin, we examined genetic interactions between *pom152* mutants and *nup1Δ*. A *nup1* deletion is lethal in combination with *pom152Δ*, as well as with truncations of the N-terminal and transmembrane regions of Pom152p. However, truncations of the N-glycosylated, luminal domain of Pom152p and *pom152* mutants lacking N-linked glycosylation sites are viable in combination with *nup1Δ*, suppress *nup1Δ* temperature sensitivity, and partially suppress the nuclear protein import defects associated with the deletion of *NUP1*. These data provide compelling evidence for a role for glycosylation in influencing NPC function.

THE nuclear envelope (NE) of eukaryotic cells is composed of two concentric lipid bilayers, the inner nuclear membrane (INM) and the outer nuclear membrane (ONM). The ONM is contiguous with the endoplasmic reticulum (ER) and the space between the ONM and INM is equivalent to the ER lumen. The only site of contact between the ONM and INM is at the nuclear pore complexes (NPCs). NPCs form aqueous channels between the cytoplasm and nucleus, allowing passive diffusion across the NE of ions and other small molecules and regulated transport of larger molecules, including proteins and mRNAs. NPCs are composed of >30 different proteins termed nucleoporins (Nups), which are generally present in 8–32 copies per NPC (reviewed in SUNTHARALINGAM and WENTE 2003).

Nup proteins provide both the structure and the transport function of NPCs. Most Nups occupy a discrete location along the nuclear/cytoplasmic axis within the NPC, with some Nups asymmetrically local-

ized toward the cytoplasmic face, some Nups at the nucleoplasmic face, and some distributed symmetrically within the central core of the NPC (ROUT *et al.* 2000). This asymmetry may allow specific Nups to function in specific stages of substrate translocation across the NPC (PYHTILA and REXACH 2003; ROUT *et al.* 2003). Nups can be divided into two basic classes based on the presence or the absence of a conserved phenylalanine-glycine (FG) repeat sequence. The FG repeats function as low-affinity docking sites for soluble transport factors that facilitate the translocation across the NPC of cargoes containing nuclear localization signals (NLSs) or nuclear export signals. These transport factors, which include the karyopherin/importin/exportin family of Ran-binding proteins, associate directly with FG repeats and are dependent upon this association for translocation across the NPC (see FAHRENKROG *et al.* 2004).

FG Nups can be divided further into subclasses based on composition of their FG repeats. The cytoplasmically oriented Nup42p and Nup159p proteins contain conserved SAFG or PSFG sequences within each repeat (GORSCH *et al.* 1995; STUTZ *et al.* 1995). These FG repeats associate with soluble transport factors, but are not essential for cell viability, despite their asymmetric distribution within the NPC (KRAEMER *et al.* 1995; DEL PRIORE *et al.* 1997; HURWITZ *et al.* 1998; FLOER and

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BLOBEL 1999; STRAWN *et al.* 2004). GLFG-Nups contain a glycine-leucine pair upstream of most FGs and seem to have the broadest substrate specificity. GLFG repeat sequences associate with most karyopherins (Kaps) and are localized symmetrically within the central region of the NPC (ROUT *et al.* 2000; ALLEN *et al.* 2001). Removal of the GLFG region of individual GLFG-Nups in *Saccharomyces cerevisiae* does not affect cell viability or transport kinetics, but the disruption of specific combinations of GLFG regions from two or more GLFG-Nups results in lethality and/or decreased nuclear import rates (STRAWN *et al.* 2004). Thus, some combination of GLFG repeats is necessary for efficient NPC function. FxFG repeats are found in Nup1p, Nup2p, Nup60p, and Nsp1p in yeast. These repeats associate with the essential heterodimeric karyopherin Kap95p/Kap60p, which functions to import classical NLS (cNLS)-containing proteins. Interestingly, cells lacking FxFG repeats from all four of these proteins are viable (STRAWN *et al.* 2004) and a Nup1p domain without FGs also associates with Kap95p/Kap60p (PYHTILA and REXACH 2003), suggesting that the FxFG repeats are not essential for Kap95/Kap60-mediated protein import.

While FxFG repeats are not required for cell viability in yeast, the FxFG-repeat-containing Nup1 protein is essential in some genetic backgrounds (DAVIS and FINK 1990) and a loss of Nup1 is lethal at elevated temperatures in most others (BOGERD *et al.* 1994; SCHLAICH and HURT 1995). Complete disruptions of *NUP1* are also synthetically lethal with mutant alleles of *NUP2* and *NSP1* (LOEB *et al.* 1993), suggesting that the non-FG sequences of these Nups may carry out important overlapping functions. Nup1, Nup2, and Nup60 are localized to the nucleoplasmic face of the NPC where they associate transiently with the NPC basket, while Nsp1 is associated with the central region of the NPC (ROUT *et al.* 2000). *NUP1* deletion (*nup1Δ*) mutants are unable to efficiently export poly(A) RNA or to import cNLS-containing cargoes at elevated temperatures (BOGERD *et al.* 1994; SCHLAICH and HURT 1995; FISCHER *et al.* 2002). However, the role of Nup1p in these processes remains to be determined.

While many Nups contain FG repeats, over half of the nucleoporins do not exhibit FG repeat sequences. Both metazoan and yeast cells express non-FG-Nups that are integral membrane proteins, associating with the NPC via a cytoplasmic region, spanning the NE, and containing a domain within the NE lumen. The presence of a transmembrane domain allows these proteins to provide a physical link between the NPC and NE (see SUNTHARALINGAM and WENTE 2003). In metazoans, gp210 is a membrane-spanning glycoprotein essential for NPC assembly (WOZNAK *et al.* 1989; COHEN *et al.* 2003). The majority of gp210 is on the cisternal side of the NPC pore membrane, with only a short 60-amino-acid region exposed on the NPC side of the membrane (GREBER *et al.* 1990). Both overexpression of this tail

domain and RNAi-mediated depletion of gp210 inhibit NPC assembly (DRUMMOND and WILSON 2002; COHEN *et al.* 2003). Expression of antibodies that associate with the luminal domain of gp210 results in decreased nuclear import of NLS-containing proteins and a reduced diffusion rate across the pore of smaller proteins, implicating the glycosylated region of gp210 in NPC function (GREBER and GERACE 1992). However, the role of glycosylation in NPC assembly and nucleocytoplasmic transport has not been examined.

In yeast, three transmembrane NPC proteins—Ndc1p, Pom34p, and Pom152p—have been identified and all three lack FG repeats. Ndc1p is a multipass transmembrane protein that localizes to both NPCs and the spindle pole body (CHIAL *et al.* 1998). Ndc1p function at the NPC is important for NPC assembly, but *ndc1* mutants have not been observed to affect transport activity (LAU *et al.* 2004). Pom34p is a small, transmembrane NPC protein of unknown function (ROUT *et al.* 2000). Pom152p, like gp210 in mammalian cells, is a large, transmembrane glycoprotein with the majority of its mass in the NE lumen (WOZNAK *et al.* 1994; TCHEPEREGINE *et al.* 1999). Despite encoding the only large integral membrane protein in the yeast NPC, *POM152* is not essential (WOZNAK *et al.* 1994). However, truncation mutants of *POM152* lacking NPC and transmembrane domains exhibit synthetic lethal interactions with genes encoding several other NPC components, including Nup188p, Nup157p, Nup170p, Nup59p, and Nic96p (ARTCHISON *et al.* 1995; TCHEPEREGINE *et al.* 1999). Of these Nups, only *nup188* mutants also exhibit synthetic interactions with a truncation of the glycosylated luminal domain of *POM152* (TCHEPEREGINE *et al.* 1999). The function of the Pom152p luminal domain has not been determined. Here we provide genetic and functional evidence that the glycosylation of Pom152 influences NPC function.

MATERIALS AND METHODS

Reagents, strains, plasmids, and media: Enzymes for molecular biology were purchased from New England Biolabs (Beverly, MA) and Sigma-Aldrich (St. Louis) and were used per manufacturer's instructions. Yeast transformations were performed as described (WOODS and GIETZ 2001) as were genetic manipulations, yeast cell culture, and media preparation (GUTHRIE and FINK 1991). Tunicamycin suppression was tested on YPD plates containing 0.5 or 1.0 $\mu\text{g/ml}$ tunicamycin (Sigma-Aldrich). Plasmids pPom152-HA, p1-301, p170-1337, and pΔ176-263 were kindly contributed by R. Wozniak (University of Alberta). Plasmid KBB382 (*CEN LEU2 pom152Δglyc*) was generated by site-directed mutagenesis of pPom152-HA using oligonucleotides that altered codons encoding asparagines at residues 280, 398, 569, and 1099 to alanine. Mutagenesis was performed using the QuikChange multi-mutagenesis kit (Stratagene, La Jolla, CA) per manufacturer's instructions. Mutagenesis was confirmed by DNA sequencing using standard techniques. Yeast strains containing genomic deletions of *NUP1*, *NUP188*, *NUP100*, *POM152*, and *ALG12* were purchased

TABLE 1
Yeast strains used in this study

Strain name	Genotype	Source
BY4742	<i>MATα his3 leu2 lys2 ura3</i>	Open Biosystems
W303	<i>MATα ade2 trp1 leu2 his3 ura3</i>	R. Rothstein
Y-728	<i>MATα nic96-1::ProtA::LEU2 nic96ΔHIS3 ura3 ade2 trp1</i>	ZABEL <i>et al.</i> (1996)
SWY29	<i>MATα nup116-5ΔHIS3 ade2 ura3 his3 trp1 leu2</i>	WENTE <i>et al.</i> (1992)
NUP82- Δ 108	<i>MATα nup82ΔHIS3 leu2 lys2 trp1 ura3 + (pNup82Δ108 LEU2)</i>	HURWITZ and BLOBEL (1995)
LGY101	<i>MATα rat7-1/nup159-1 his3 ura3 leu2</i>	GORSCH <i>et al.</i> (1995)
LDY461	<i>MATα nup1ΔLEU2 ade2 trp1 leu2 his3 ura3</i>	This study
LDY563	<i>MATα nup1ΔLEU2 nup100ΔURA3 trp1 his3 ade2</i>	This study
LDY572	<i>MATα nup100ΔURA3 leu2 ade2 his3 trp1</i>	This study
LDY796	<i>MATα nup1ΔHIS3 ade2 trp1 leu2 ura3</i>	This study
KBY353	<i>MATα bnp1::Tn3::LEU2 nup1ΔHIS3 ade2 trp1 leu2 ura3</i>	This study
KBY423	<i>MATα nup1ΔHIS3 ade2 trp1 leu2 ura3</i>	This study
KBY644	<i>MATα nup133ΔKAN his3 leu2 lys2 ura3</i>	Open Biosystems
KBY669	<i>MATα nup1ΔHIS3 ade2 ura3 trp1</i>	This study
KBY671	<i>MATα nup1ΔHIS3 ecm39ΔKANR ade2 ura3 trp1</i>	This study
KBY673	<i>MATα ecm39ΔKAN ura3 trp1</i>	This study
KBY675	<i>MATα ura3 his3 trp1</i>	This study
KBY776	<i>MATα pom152ΔKAN his3 leu2 ura3 lys2</i>	Open Biosystems
KBY795	<i>MATα nup170ΔKAN his3 leu2 lys2 ura3</i>	Open Biosystems
KBY1046	<i>MATα pom152ΔKAN his3 leu2 ura3 lys2+ (LDB59 CEN URA3 NUP1)</i>	This study
KBY1047	<i>MATα nup1ΔKAN pom152ΔKAN his3 leu2 ura3 + (LDB59 CEN URA3 NUP1)</i>	This study
KBY1048	<i>MATα nup1ΔKAN his3 leu2 ura3 + (LDB59 CEN URA3 NUP1)</i>	This study
KBY1293	<i>MATα nup188ΔKAN his3 leu2 ura3 lys2</i>	Open Biosystems
KBY1329	<i>MATα pom152ΔKAN his3 leu2 ura3 lys2+ (KBB382 LEU2 pom152Δglyc)</i>	This study
KBY1366	<i>MATα pom152ΔKAN his3 leu2 ura3 lys2+ (pRS315 CEN LEU2)</i>	This study
KBY1374	<i>MATα nup1ΔKAN pom152ΔKAN his3 leu2 ura3 lys2+ (KBB382)</i>	This study

from OpenBiosystems (Huntsville, AL) and mated to produce strains used in this study (Table 1).

bnp suppressor screen: Yeast strain LDY796 (Table 1) was transformed with a *NotI*-digested mTn::LEU2 mutagenized yeast library, as described in ROSS-MACDONALD *et al.* (1997), and transformants were plated on SC –Leu media. Transformants were replica plated to SC –Leu media containing 1 mg/ml 5-fluoro-orotic acid (5-FOA) and incubated at 37°. Viable colonies were restreaked, mated to a W303 wild-type haploid, sporulated, and dissected. Resulting haploid spores were scored for cosegregation of Leu⁺ ts⁺ phenotypes. The genomic DNA immediately adjacent to the Tn insertion in *bnp1* was identified using isolation of an integrated copy of the pRSQ2-URA plasmid as described previously (ROSS-MACDONALD *et al.* 1997) followed by sequencing of the isolated plasmid DNA. The presence of the transposon within genomic *ALG12* was confirmed by PCR using primer pairs internal to the transposon and to *ALG12* (data not shown).

Protein import and RNA export: To examine protein import, strains KBY675 (wild type), KBY669 (*nup1 Δ*), KBY671 (*alg12 Δ nup1 Δ*), KBY673 (*alg12 Δ*), BY4742 (wild type), KBY1366 (*pom152 Δ*), KBY1329 (*pom152 Δ glyc*), and KBY1374 (*nup1 Δ pom152 Δ glyc*) were transformed with plasmid pSV40-NLS-GFP (SHULGA *et al.* 1996), grown in SD –Ura to A₆₀₀ 0.05–0.2, and observed by direct fluorescence microscopy using a Nikon E600 epifluorescence microscope. Images were captured using a Hamamatsu (Tokyo) C5810 cooled CCD camera using National Institutes of Health Image software and were processed using Adobe Photoshop. *In situ* hybridization assays were performed on strains LDY675, KBY669, KBY671, and KBY673 as described previously (AMBERG *et al.* 1992).

Analysis of *pom152* mutants in *nup1 Δ* : Plasmids expressing truncated alleles of *POM152* (pPom152-HA, p1-1219, p1-301

p170-1337, p1025-HA, and p Δ 295-1036-HA; TCHEPEREGINE *et al.* 1999) and KBB382 were transformed into strain KBY1047 [*nup1 Δ ::KAN^R pom152 Δ ::KAN^R + pLDB59 (CEN URA3 NUP1)] and transformants were selected for on SC –Leu media. Transformants were streaked to SC –Leu media containing 1 μ g/ml 5-FOA to select against pLDB59 (BOEKE *et al.* 1987) and to SC –Ura media. Cells were incubated at 24°, 30°, and 37° for 2–5 days and scored for growth.*

Protein extraction and Western blotting: Yeast strains BY4742 or KBY673 containing pPom152-HA or KBB382 were grown to OD₆₀₀ = 0.2–0.4 in 10 ml SD –Leu media. Cells were then lysed using acid-washed glass beads and precipitated using trichloroacetic acid as described (ADAMS *et al.* 1997). Cells treated with tunicamycin were incubated in SD –Leu containing 1.0 μ g/ml tunicamycin for 3 hr before lysis. Protein extracts were electrophoretically separated on 7.5% polyacrylamide, transferred to nitrocellulose, and probed with 12CA5 anti-HA antibody (Babco, Berkeley, CA). Detection of bound antibody was performed using chemiluminescence (Bio-Rad, Hercules, CA).

RESULTS

Cells lacking functional Nup1p have a temperature-sensitive growth phenotype and exhibit defects in mRNA nuclear export, nuclear protein import, and NE morphology (DAVIS and FINK 1990; BOGERD *et al.* 1994; SCHLAICH and HURT 1995). While *nup1 Δ* cells are dead at 37° and grow slowly at 25°, we observed that *nup1 Δ* cells grown under either of these conditions

FIGURE 1.—A *nup100* deletion suppresses *nup1Δ* temperature sensitivity. Wild type (W303), *nup100Δ* (LDY572), *nup1Δ* (LDY461), and *nup1Δ nup100Δ* (LDY563) haploid cells were grown in liquid culture to midlog phase and fivefold serial dilutions were spotted on YPD. Plates were incubated at 25° for 3 days and at 37° for 2 days.

spontaneously accumulate colonies in which the conditional or slow-growth phenotype is suppressed (Figure 1; see *nup1Δ* cells at 37°). These suppressors are not linked to *NUP1* and arise at high frequency, suggesting that the reversions are due to loss-of-function mutations in loci distinct from *NUP1*. We have observed this “suppressor” phenotype in *nup1Δ*-deleted cells from several different strain backgrounds (data not shown). One possible explanation for the frequent appearance of *nup1Δ* suppressors is that the loss of any one of a large number of other genes could allow the normal cellular requirement for *NUP1* to be lost, effectively allowing the cell to bypass *NUP1* function. To test whether decreased function of another NPC component could bypass the normal *Nup1p* requirement at elevated temperature, we generated double-mutant cells mutated for *NUP1* and other Nups. Mutations in *NSP1*, *NUP2*, *NUP82*, *NUP116*, *NUP157*, and *NUP170* are synthetically lethal with *nup1Δ* (LOEB *et al.* 1993; KENNA *et al.* 1996; SHULGA *et al.* 1996; data not shown). However, *nup1Δ nup100Δ* cells grew better at 25° than *nup1Δ* cells and grew at a rate indistinguishable from wild type at 37° (Figure 1). We observed this suppression of *nup1Δ* by *nup100Δ* in both W303 and S288C yeast strain backgrounds (data not shown). Thus, a deletion of a nucleoporin can bypass the *NUP1* requirement for cell viability at 37°.

A deletion in *ALG12* suppresses *nup1Δ* temperature sensitivity: The high frequency at which suppressors spontaneously appeared in *nup1Δ* cells suggested that other loss-of-function mutations would also be able to bypass *nup1Δ* growth phenotypes. To identify other mutations that bypass *nup1Δ*, we performed a large-scale genetic screen for second-site mutations that suppress *nup1Δ* temperature sensitivity (Figure 2A). To this end, we performed random mutagenesis on a *nup1Δ* strain by insertion of a transposon 3 (Tn3)-disrupted yeast genomic library (ROSS-MACDONALD *et al.* 1997). We then assayed the colonies receiving Tn3-containing DNA insertions for viability at 37°, which would indicate suppression of the temperature-sensitive phenotype of the *nup1Δ* parent strain. Those Tn3-containing strains that grew at 37° were backcrossed with the *nup1Δ* parent strain and the resulting diploids

were dissected to confirm linkage of a single transposon with the suppressor phenotype in each mutant (see MATERIALS AND METHODS). In this way, we identified 32 bypass suppressor of *nup1Δ* (*bnp*) mutants, which we have labeled *bnp1*–*bnp32*.

Here we report the characterization of the *bnp1* transposon insertion mutant. In this isolate, the presence of the transposon suppressed the temperature sensitivity of the *nup1Δ* deletion, restoring growth at 37° to nearly the rate of wild-type cells (Figure 2B). To identify the gene disrupted by the transposon in the *bnp1* strain, we isolated DNA adjacent to the site of transposon insertion using plasmid rescue (ROSS-MACDONALD *et al.* 1997) and sequenced the DNA of the rescued, Tn-containing plasmid. In our *bnp1* mutant, the transposon had inserted within the coding region of the *ALG12* gene, resulting in truncation of the Alg12 protein after only 30 amino acids. Thus *bnp1* is a severe truncation allele of *ALG12* (ALG, asparagine-linked glycosylation; BURDA *et al.* 1999). *ALG12* was first identified in a screen for cells resistant to calcofluor (LUSSIER *et al.* 1997) and encodes an ER-localized α -16-mannosyl-transferase required for synthesis of Man₈GlcNAc₂ from Man₇GlcNAc₂ (BURDA *et al.* 1999).

Although the mutagenic transposon is inserted within the *ALG12* gene, a small region of the endogenous Alg12p may still be produced. To confirm that the suppression of *nup1Δ* that we observed in *bnp1* was the result of an *alg12* loss-of-function mutation, we mated *nup1Δ* and *alg12Δ* haploid cells containing complete disruptions of each gene. We then sporulated and dissected the heterozygous diploids to isolate *nup1Δ alg12Δ* double-mutant haploid cells and compared the growth of *alg12Δ nup1Δ* cells with *alg12Δ* and *nup1Δ* single mutants (Figure 2C). Both wild-type and *alg12Δ* cells grew at similar rates at all temperatures tested, while *nup1Δ* cells grew slowly at 25° and were inviable at 37°. Importantly, *nup1Δ alg12Δ* double mutants grew at a rate indistinguishable from wild type at 25° and significantly better than *nup1Δ* at 37°. These data indicate that a loss-of-function *alg12* mutant can function as a bypass suppressor of *nup1Δ* temperature sensitivity.

***alg12Δ* suppresses the nuclear protein import defect exhibited in *nup1Δ*:** *Nup1p* is essential both for efficient nuclear import of cNLS-containing proteins and for export of polyadenylated RNAs (DAVIS and FINK 1990; BOGERD *et al.* 1994; SCHLAICH and HURT 1995; FISCHER *et al.* 2002). Because an *alg12Δ* mutation can suppress the growth phenotype associated with a deletion of *nup1*, we investigated whether *alg12Δ* mutants influence nuclear transport kinetics, both in cells that are otherwise wild type and in *nup1Δ* cells. To examine whether protein import is altered in *alg12Δ* and *nup1Δ alg12Δ* cells, we examined the intracellular localization of green fluorescent protein (GFP)-tagged nuclear import substrates in *alg12Δ* mutant cells. As a nuclear import substrate, we used cNLS-GFP, containing a “classical”

FIGURE 2.—Mutations in *ALG12* suppress *nup1Δ* temperature sensitivity. (A) Flow diagram describing the genetic screen used to identify bypass suppressors of *nup1Δ* (*bnp*'s). (B) Bypass suppressors of *nup1Δ* temperature sensitivity (*bnp*'s) were generated as described in MATERIALS AND METHODS. Wild type (W303), *nup1Δ* (LDY796), and *nup1Δ bnp1* (KBY353) cells were streaked to YPD at 30° and at 37° and incubated for 54 hr. *bnp1* is a truncation allele of *ALG12* generated by Tn mutagenesis. (C) Log-phase cultures of wild type (W303), *alg12Δ* (KBY673), *nup1Δ* (KBY669), and *nup1Δ alg12Δ* (KBY671) cells were serially diluted and plated on YPD. Images were taken after 96 hr at 25° and after 56 hr at 37°.

NLS imported by the Kap95p/Kap60p heterodimer (SHULGA *et al.* 1996). Nup1p associates directly with Kap95p/Kap60p and is essential for efficient cNLS import (BOOTH *et al.* 1999; PYHTILA and REXACH 2003). We expressed cNLS-GFP in wild-type, *nup1Δ*, *alg12Δ*, and *nup1Δ alg12Δ* cells and observed the steady-state localization of the cNLS-GFP reporter in live cells by fluorescence microscopy. Cells were either incubated at 25° or shifted from 25° to 37° for 3 hr before assaying. All four strains exhibited predominantly nuclear accumulation of cNLS-GFP at 25° (Figure 3A). However, both *nup1Δ* and *nup1Δ alg12Δ* cultures contained some cells (<25%) with predominantly cytoplasmic GFP. In cells shifted to 37°, nearly all of the wild-type and *alg12Δ* cells accumulated nuclear cNLS-GFP, while the *nup1Δ alg12Δ* double mutant again had >70% of cells with nuclear fluorescence. Only the *nup1Δ* cells showed a severe defect in nuclear cNLS-GFP import, with <10% of cells accumulating GFP within the nucleus. These observations suggest that a deletion of *alg12* can suppress the severe cNLS import defect exhibited by *nup1Δ* cells, providing a possible mechanism by which *alg12Δ* suppresses *nup1Δ* temperature sensitivity.

While these data indicate that *alg12Δ nup1Δ* cells can import cNLS-GFP, they do not address the relative rate of cNLS nuclear import in each strain. To determine if an *alg12* deletion alters the rate of cNLS-GFP import, we performed a kinetic nuclear import assay (SHULGA *et al.*

1996). At 25°, both wild type and *alg12Δ* cells imported the cNLS-GFP reporter at equivalent rates, with ~50% of cells exhibiting nuclear cNLS-GFP accumulation within 11 min after removal of metabolic inhibitors that effectively abolish active nucleocytoplasmic transport (Figure 3B). In contrast, only ~20% of *nup1Δ* cells accumulated the cNLS reporter in the nucleus after an equivalent amount of time. This slowed rate of cNLS-mediated import was partially suppressed by a deletion of *ALG12*, as 42% of *alg12Δ nup1Δ* cells accumulated nuclear cNLS-GFP after 11 min. At 37°, *alg12Δ* cells exhibited slightly slower cNLS import kinetics than did wild-type cells, with ~5 min required to accumulate nuclear fluorescence in 50% of *alg12Δ* cells compared to 3.5 min in wild type. In contrast, *nup1Δ* cells nearly entirely lacked cNLS import with <20% of cells accumulating nuclear cNLS-GFP, even 30 min after removal of metabolic inhibitors. *alg12Δ nup1Δ* cells had an intermediate rate of cNLS-GFP import, with 50% of cells containing nuclear GFP after ~17 min. Thus, deletion of *ALG12* partially suppresses the cNLS-GFP import defect associated with the loss of *NUPI*.

Cells lacking Nup1p also exhibit a reduction in nuclear poly(A)⁺ RNA export (BOGERD *et al.* 1994; SCHLAICH and HURT 1995; FISCHER *et al.* 2002). To test for the influence of *alg12* deletions on mRNA export, we examined poly(A)⁺ RNA localization in cells

FIGURE 3.—Deletion of *ALG12* suppresses *nup1Δ* protein import defects but not poly(A)⁺ RNA export defects. (A) Wild-type (KBY675), *nup1Δ* (KBY669), *alg12Δ* (KBY673), and *nup1Δ alg12Δ* (KBY671) cells expressing a cNLS fused to GFP (SHULGA *et al.* 1996) were grown to early log phase at 25° and then retained at 25° or shifted to 37° for 3 hr. Cells were observed by direct fluorescence (cNLS-GFP) or differential interference contrast microscopy. (B) Wild-type (KBY675), *nup1Δ* (KBY669), *alg12Δ* (KBY673), and *nup1Δ alg12Δ* (KBY671) cells were examined for cNLS-GFP import kinetics (SHULGA *et al.* 1996; see MATERIALS AND METHODS). Briefly, cells expressing cNLS-GFP were treated with 2-deoxyglucose and sodium azide for 1 hr, washed, and then assayed for cNLS reimport by fluorescence microscopy. Import rate was determined by plotting the percentage of cells exhibiting nuclear fluorescence of cNLS-GFP *vs.* time. (Top) Relative cNLS-GFP import rates in cells grown at 25°. (Bottom) Cultures shifted to 37° for 3 hr and retained in 37° media throughout the import assay. Data represent mean values from at least three independent experiments. Error bars represent standard error of the mean. (C) Wild-type, *nup1Δ*, *alg12Δ*, and *nup1Δ alg12Δ* cells were grown to early log phase at 24° and then incubated at 24° or shifted to 36° for 3 hr. Cells were fixed, permeabilized, and incubated with a digoxigenin-conjugated oligo(dT)₅₀ probe. Hybridized probe was detected using FITC-associated antidigoxigenin antibodies (AMBERG *et al.* 1992). “Poly(A)” represents localization of polyadenylated RNA, as detected by the oligo(dT) probe. “DAPI” indicates the location of DAPI-stained nuclei in the same cells.

lacking Alg12p using *in situ* hybridization with an oligo(dT) probe (AMBERG *et al.* 1992). None of the *nup1Δ*, *alg12Δ*, or *nup1Δ alg12Δ* mutant cells exhibited an accumulation of poly(A)⁺ RNA in the nucleus when grown at the permissive temperature (Figure 3C). However, after a 3-hr shift to 36°, some *nup1Δ* cells exhibited clear nuclear poly(A)⁺ RNA localization, in agreement with the partial RNA export defect observed previously for *nup1* mutants (BOGERD *et al.* 1994; SCHLAICH and HURT 1995; FISCHER *et al.* 2002). *nup1Δ alg12Δ* double-mutant cells also exhibited a

nuclear accumulation of poly(A)⁺ RNA in the nucleus, similar to that seen for *nup1Δ*. In contrast, wild-type and *alg12Δ* cells did not accumulate poly(A)⁺ RNA in the nucleus. These data suggest that the deletion of *alg12Δ* does not suppress the mRNA export defect of *nup1Δ* cells.

Decreased glycosylation suppresses *nup1Δ* temperature sensitivity: Alg12p is a mannosyltransferase required for normal assembly of lipid-associated polysaccharide chains in the ER. Once assembled, these branched chains are then transferred to specific

FIGURE 4.—Inhibition of glycosylation alters the growth rate of *nup1*, *nup116*, *nup82*, and *nic96* cells. (A) Wild-type (W303) and *nup1Δ* (KBY423) cells were grown to log phase in YPD at 25° and then serially diluted and plated on YPD and YPD containing 0.5 μg/ml tunicamycin. Plates were incubated at 30° for 72 hr. (B) Overnight cultures of wild-type (W303 and BY4742), *nup170Δ* (KBY795), *nup133Δ* (KBY644), *rat7-1/nup159^{ts}* (LGY101), *nup188Δ* (KBY1293), *nup116Δ* (SWY29), *nic96-1* (Y-728), and *nup82Δ108* (NUP82-Δ108) cells were grown to log phase, resuspended at identical concentrations, serially diluted, and plated on YPD (–Tunic) or on YPD containing 1.0 μg/ml tunicamycin (+Tunic). Plates were incubated at 24° and 32.5° for 72 hr. (C) Wild-type (BY4742) and *nup1Δ* (LDY461) cells were transformed with pGAL::SSA1 (2μ *LEU2* GAL::SSA1) or pRS315 (*CEN* *LEU2*). Cells were grown to log phase and serial dilutions were spotted onto YPD media containing glucose or galactose. Plates were incubated at 32.5° for 72 hr.

asparagine (N) residues on proteins within the ER lumen. This “N-linked” glycosylation occurs on most proteins that enter the ER and the presence of these branched carbohydrate chains is essential for the stability, localization, and function of many glycosylated proteins (reviewed in HELENUS and AEBI 2001). Since Alg12p is important for normal glycosylation, we tested to see if inhibiting glycosylation *in vivo* would alter the phenotypes associated with mutations in *NUP1*. To this end, we plated serially diluted cultures containing *nup1Δ* and wild-type cells on tunicamycin, an inhibitor of protein glycosylation (MAHONEY and DUKSIN 1979), and assayed for growth at temperatures ranging from 24° to 37°. We observed that both wild-type and *nup1Δ* cells failed to grow at any temperature in the presence of 2.0 μg/ml tunicamycin (data not shown). However, cells exposed to lower doses of tunicamycin remained viable but had slowed growth kinetics. We observed that wild-type cells grown at 30° for 48 hr in the presence of 0.5 μg/ml tunicamycin exhibited a slightly decreased growth rate compared to cells incubated on YPD (Figure 4A). In contrast, *nup1Δ* cells incubated under the same conditions grew significantly better in the presence of tunicamycin than on YPD. A similar increase in growth in the presence of 0.5 μg/ml tunicamycin was observed for *nup1Δ* cells incubated at 28° and 32.5° (data not

shown). Thus, a general decrease in glycosylation *in vivo* partially suppresses the temperature-sensitive phenotype of *nup1Δ* cells.

To determine if the glycosylation-dependent growth defects that we observed were specific to *nup1Δ*, we incubated additional NPC mutants on media containing tunicamycin. A tunicamycin concentration of 1.0 μg/ml strongly suppressed the conditional growth of *nup82Δ108* at 32.5° (Figure 4B) and at 37° (data not shown), but did not significantly alter the growth of *nup133Δ*, *nup188Δ*, *nup159^{ts}*, *nup100Δ*, *nup2Δ*, *nup53Δ*, or *nup59Δ* cells compared to wild type (Figure 4B; data not shown). Interestingly, *nup116Δ*, *nic96-1*, and *nic96-2* (Figure 4B; data not shown) mutants exhibited hypersensitivity to tunicamycin. These data suggest that glycosylation influences a distinct subset of Nups and that different Nup proteins may have distinct functional interactions with glycosylated proteins.

The inhibition of glycosylation by tunicamycin has been shown previously to induce the unfolded protein response (UPR) for elimination of misfolded ER proteins (TRAVERS *et al.* 2000). One consequence of UPR induction is an increase in expression of some protein chaperones and their cofactors. Overexpression of Ssa1p, a cytosolic yeast hsp70, has been shown previously to suppress defects in cNLS-mediated

FIGURE 5.—*nup1Δ* growth defects are suppressed by *pom152* alleles lacking glycosylation. (A) Wild-type (S288C), *pom152Δ* (KBY1046), *nup1Δ* (KBY1048), and *pom152Δ nup1Δ* (KBY1047) cells containing pLDB59 (*CEN URA3 NUP1*) were streaked on SD –Ura and 5-FOA media, incubated at 25° for 4 days, and scored for growth. (B) *nup1Δ pom152Δ* (KBY1047) cells containing a *CEN URA3 NUP1* plasmid were transformed with plasmids expressing full-length *POM152*, deletions of N-terminal (p170-1337), transmembrane (pΔ176-263), and luminal (p1-301) Pom152 domains (TCHEPEREGINE *et al.* 1999), and empty vector (pRS315). Transformants were streaked on SD –Leu and 5-FOA and incubated at 24°. Streaks were scored for growth after 3 days on SD –Leu and after 5 days on 5-FOA. (C) *POM152*, *pom152Δ Glyc* (KBB382), *pom152Δ C* (p1-301), and empty vector (pRS315) were transformed into *nup1Δ pom152Δ* (KBY1047) cells containing a *CEN URA3 NUP1* plasmid. Transformants were streaked to SD –Leu and 5-FOA and incubated at 24° and at 37°.

nuclear import (SHULGA *et al.* 1996, 1999). To ascertain whether the suppression of *nup1Δ* temperature sensitivity that we observed was the result of increased Ssa1p, we overexpressed *SSA1* in wild-type and *nup1Δ* cells and assayed for growth at permissive and non-permissive temperatures (Figure 4C). The *nup1Δ* cells did not exhibit a detectable change in growth at either temperature upon overexpression of Ssa1p, suggesting that suppression of *nup1Δ* by tunicamycin is not simply due to an UPR-induced increase in Ssa1p activity.

***nup1Δ* cells exhibit allele-specific interactions with *pom152* mutants lacking N-linked glycosylation sites:** Since N-linked glycosylation occurs in the ER lumen, most NPC proteins are unlikely to be glycosylated. However, transmembrane proteins that link the NPC to the NE span the lipid bilayer and extend into the luminal space between the inner and outer nuclear membranes. This space is contiguous with the ER lumen, allowing transmembrane Nups to be glycosylated on their luminal domains. Indeed, Pom152p is a

type II transmembrane nucleoporin whose C-terminal domain is within the NE lumen and is glycosylated at from one to four asparagine residues (TCHEPEREGINE *et al.* 1999). Given the link between glycosylation and Nup1p function that we had observed, we examined whether *nup1* and *pom152* mutants exhibited genetic interactions. To this end, we generated wild-type, *pom152Δ*, *nup1Δ*, and *pom152Δ nup1Δ* haploid mutant cells containing a *NUP1 URA3* plasmid (Figure 5A). We then tested each strain for growth on SD –Ura media, in which the plasmid-borne *NUP1* is retained, and on 5-FOA, which selects against the *URA3*-containing *NUP1* plasmid. A deletion of *POM152* is not lethal and does not confer a detectable growth phenotype (WOZNIAK *et al.* 1994). As expected, cells lacking *NUP1* exhibited a temperature-sensitive phenotype, growing slowly at 25°, but failing to grow at 37°. Surprisingly, cells lacking both *NUP1* and *POM152* were inviable at all temperatures. Thus, *nup1Δ pom152Δ* mutants are synthetically lethal, suggesting a functional interaction between the two gene products.

The synthetic lethality between *nup1Δ* and *pom152Δ* was somewhat unexpected, given our observation that a decrease in glycosylation actually increases growth rates of *nup1Δ* mutants. We predicted that the synthetic lethality between *NUP1* and *POM152* was the result of the dependence of *nup1Δ* mutants on a region of Pom152p other than the glycosylated residues. Other Nups that are synthetically lethal with *pom152* exhibit allele-specific synthetic interactions with *pom152* mutants lacking specific sequences (TCHEPEREGINE *et al.* 1999). To determine if *nup1Δ* is synthetically lethal with specific deletions of Pom152p domains, we tested whether several *pom152* truncation alleles (TCHEPEREGINE *et al.* 1999) were viable in combination with a deletion of *nup1Δ*. To this end, *nup1Δ pom152Δ* cells containing a *URA3 NUP1* plasmid were transformed with plasmids expressing N-terminal (*p170–1337*), transmembrane domain (*pΔ176–263*), and C-terminal (*p1–301*) deletions of *POM152*. Transformants were then plated on 5-FOA-containing media to select against the *URA3 NUP1* plasmid and were scored for growth at 24° and 37° (Figure 5B). At 24° *nup1Δ* cells containing N-terminal or transmembrane deletions of *POM152* failed to grow, indicating that cells lacking Nup1p require both the NPC domain and the transmembrane domain of Pom152p for viability. However, *nup1Δ* cells expressing a *pom152* mutant lacking the C-terminal 1036 residues (*p1–301*, hereafter referred to as *pom152ΔC*) grew at a rate indistinguishable from *nup1Δ* cells expressing full-length Pom152p. These data indicate that only the N-terminal 301 amino acids of Pom152p, including the NPC and transmembrane domains, are essential for complementation of *nup1Δ pom152Δ* synthetic lethality while the luminal C-terminal domain is not. At 37°, the N-terminal and transmembrane truncations were again inviable in a *nup1Δ* background (data not shown). As expected, *nup1Δ* cells expressing full-length *POM152* were also inviable, since the *nup1Δ* is temperature sensitive. However, cells expressing a C-terminal truncation of Pom152p grew at 37° in the absence of Nup1p (Figure 6B), indicating that the loss of the luminal, glycosylated Pom152p domain can suppress the temperature sensitivity of a *nup1* deletion.

The suppression of *nup1Δ* by *pom152ΔC* may be the consequence of removing Pom152p glycosylation sites or the result of truncating an important Pom152p polypeptide sequence. Pom152p is glycosylated on Asp280 and at up to three other luminal asparagines (WOZNIAK *et al.* 1994; TCHEPEREGINE *et al.* 1999). On the basis of the consensus glycosylation sequence NXS/T, potential glycosylation sites also exist at N398, N569, and N1099 within the luminal domain of Pom152p. To determine if a *pom152* allele lacking glycosylation confers a *nup1Δ* suppressor phenotype, we performed site-directed mutagenesis on all four predicted N-linked glycosylation sites present in the C-terminal domain of Pom152p, converting each asparagine to an alanine. We

confirmed the generation of *pom152^{N280A, N398A, N569A, N1099A}* (*pom152Δglyc*) by DNA sequencing (data not shown) and by observing a change in electrophoretic mobility of the Pom152Δglyc protein relative to wild-type Pom152p (Figure 6A). Interestingly, Pom152p extracted from *alg12Δ* cells or cells treated with 1 μg/ml tunicamycin did not exhibit a detectable increase in electrophoretic mobility, suggesting that Pom152p retains at least a significant proportion of its glycosylated residues under these conditions.

Since *pom152Δglyc* is not glycosylated, we used this mutant to test whether the loss of Pom152p glycosylation suppresses *nup1Δ*. To this end, *pom152Δglyc* was expressed in *nup1Δ pom152Δ* cells and the growth phenotype of *nup1Δ pom152Δglyc* was compared with *nup1Δ*, *nup1Δ pom152Δ*, and wild-type cells at 24° and 37° (Figure 6B). *nup1Δ pom152Δglyc* cells grew faster than *nup1Δ* at 24°, at a rate similar to *nup1Δ pom152ΔC*. At 37°, both *nup1Δ pom152ΔC* and *nup1Δ pom152Δglyc* remained viable, while *nup1Δ* cells were unable to grow. Thus, elimination of Pom152p glycosylation suppresses the temperature-sensitive growth of *nup1Δ*.

To determine if the suppression of *nup1Δ* temperature sensitivity by Pom152Δglyc is due to enhanced nuclear protein import, we performed kinetics assays of protein import on *pom152* and *nup1* mutant cells. We first examined the rate of cNLS-GFP nuclear import in wild-type cells compared with cells lacking Pom152p and cells containing *pom152Δglyc* as their only source of Pom152p. Neither the total loss of Pom152p nor the removal of the luminal glycosylation sites from Pom152p significantly alters the nuclear import kinetics of the cNLS-GFP reporter at 24° (data not shown) or at 37° (Figure 6C). We then examined the influence of Pom152p glycosylation on nuclear import in *nup1Δ* cells. The removal of the C-terminal Pom152p glycosylation sites does not significantly alter the rate of cNLS-GFP nuclear import in *nup1Δ* cells grown at 24° (data not shown). However, the expression of Pom152Δglyc in a *nup1Δ* strain incubated at 37° increases the kinetics of cNLS-GFP import to a rate intermediate to that observed for wild-type and *nup1Δ* cells (Figure 6D). These data indicate that the loss of Pom152p luminal glycosylation alone does not significantly alter cNLS-mediated protein import under the conditions examined, but that eliminating N-linked glycosylation of the luminal domain of Pom152p does partially suppress the conditional nuclear import defect observed in *nup1Δ* mutants, thus providing a likely mechanism for the suppression of *nup1Δ* temperature sensitivity by *pom152Δglyc*.

DISCUSSION

Here we provide strong evidence that glycosylation within the lumen of the NE influences NPC function. We show that the temperature-sensitive growth

FIGURE 6.—Elimination of Pom152p glycosylation suppresses *nup1Δ* phenotypes. (A) Protein extracts from wild type and *alg12Δ* cells expressing HA-tagged Pom152Δglyc or Pom152p were run on 7.5% polyacrylamide and probed with anti-HA antibody. (Left) The electrophoretic mobility of Pom152Δglyc compared to Pom152p in each strain. (Right) The relative mobility of Pom152p extracted from cells treated with 1.0 μg/ml tunicamycin (+) vs. Pom152p from untreated (–) cells. (B) KBY1047 (*nup1Δ pom152Δ* + *CEN URA3 NUP1*) cells were transformed with plasmids containing empty vector (pRS315), *POM152* (pPom152-HA), *pom152ΔC* (p1-301), or *pom152Δglyc* (KBB382). Transformants were streaked on SD –Leu and 5-FOA and incubated at 24° and at 37°. (C) Wild-type (BY4742), *pom152Δ* (KBY1336), and *pom152Δglyc* (KBY1329) cells expressing cNLS-GFP were assayed for import of a cNLS-GFP reporter after release from metabolic arrest at 37° (SHULGA *et al.* 1996). The percentage of cells exhibiting nuclear fluorescence is plotted against the time elapsed after release from arrest. Data represent mean values from at least three independent experiments. Error bars represent standard error of the mean. (D) Wild-type (BY4742), *nup1Δ* (KBY1376), and *nup1Δ pom152Δglyc* (KBY1374) cells were examined for cNLS-GFP import kinetics as described above. Kinetic assays were performed after incubation at 37° for 3 hr.

phenotype associated with a deletion of *NUP1* is suppressed both by deletion of *ALG12*, a mannosyltransferase essential for normal glycosylation, and by treatment of cells with the glycosylation inhibitor tunicamycin. In addition, *alg12Δ* partially restores cNLS import in *nup1Δ* mutants. Finally, while *nup1Δ* is synthetically lethal with truncation of the N terminus or deletion of the transmembrane domain of Pom152p, eliminating glycosylation of the luminal C terminus of Pom152p suppresses the temperature sensitivity and nuclear transport defects of *nup1Δ*. Together, these data strongly suggest that glycosylation of the luminal domain of Pom152p plays a role in facilitating NPC function.

Despite the presence of glycosylated integral membrane proteins in the NPC, the relationship between glycosylation and NPC function remains unclear. In mammalian systems, treatment of cells with the lectin wheat germ agglutinin inhibits nuclear protein import

(FINLAY *et al.* 1987). NPCs that lack O-linked N-acetylglucosamine (GlcNAc) are unable to import karyophilic proteins or to dock Kap/cargo complexes at the cytoplasmic face of the NPC, even though the NE and major NPC structural components assemble appropriately in their absence (FINLAY and FORBES 1990). These O-linked GlcNAc residues are present on the cytoplasmic surface of FG-Nups and may play a role in substrate docking and translocation through the NPC (DAVIS and BLOBEL 1987; FINLAY and FORBES 1990; GREBER and GERACE 1992; MILLER *et al.* 1999) or may regulate the phosphorylation state of specific O-glycosylated Nups (MILLER *et al.* 1999). Interestingly, O-linked GlcNAc modification of yeast Nups has not been reported.

In contrast to O-linked modifications, N-linked glycosylation may play a global role in NPC function, as both metazoan gp210 and yeast Pom152p are

glycosylated at asparagine residues within the lumen of the NE (WOZNAK *et al.* 1989; TCHEPEREGINE *et al.* 1999). Exposure of cells to an antibody that associates with a luminal region of gp210 inhibits nuclear protein import and decreases the apparent rate of diffusion through the NPC, suggesting that the luminal domain of gp210 is essential for normal NPC function (GREBER and GERACE 1992). However, the role of glycosylation of the luminal gp210 domain in NPC function has not been examined. The importance of glycosylation in Pom152p function is equally unclear. The C-terminal glycosylated region of Pom152p is essential for viability in the absence of Nup188p, but this domain is not necessary for complementation of *pom152Δ* synthetic lethality with *nic96*, *nup170*, *nup59* (TCHEPEREGINE *et al.* 1999), or *nup1* mutants (Figure 5B), suggesting that a subset of nucleoporins may interact functionally with the luminal Pom152p region. Our observation that tunicamycin treatment alters the growth rate of some *nup* mutants [including *nup1* and *nic96* alleles, which interact genetically with *pom152* (TCHEPEREGINE *et al.* 1999; this study)], while inhibiting glycosylation does not detectably influence the growth of other *nup* deletions (including *nup170Δ* and *nup59Δ*), provides further evidence that a subset of Nups is influenced by luminal glycosylation. Together, these data provide the first strong evidence that the glycosylation state of a nucleoporin can influence NPC function.

How might glycosylation influence or regulate NPC activity to produce the phenotypes observed in our mutants? Potential alterations in NPC function manifested in these phenotypes could include changes in active transport across the NPC, changes in NPC structure, alterations in NPC assembly, or some combination of the above. Our observation that cNLS-GFP import is enhanced in *nup1Δ alg12Δ* and *nup1Δ pom152Δglyc* cells compared to *nup1Δ* cells suggests that changes in glycosylation may directly influence active transport mechanisms. These changes may be specific to nucleocytoplasmic protein transport, as the mRNA export defect observed in *nup1Δ* is not suppressed by *alg12Δ*. In metazoan cells, antibodies directed against the domain of gp210 within the NE lumen also alter transport kinetics of a nucleophilic reporter protein, resulting in a decreased nuclear import rate (GREBER and GERACE 1992). Some *sec* mutants also exhibit decreased nuclear import of cNLS-containing proteins (NANDURI *et al.* 1999), suggesting that appropriate ER function may be important for normal nucleocytoplasmic transport. Interestingly, the exposure of cells to tunicamycin does not seem to detectably alter the import kinetics of cNLS-containing reporter proteins (A. GUPTA and K. BELANGER, unpublished results).

Protein stability, intracellular targeting, and protein-protein interactions are influenced by the glycosylation state, especially within the endomembrane system (re-

viewed in HELENIOUS and AEBI 2001). Changes in glycosylation of specific nucleoporins, such as Pom152p, could thus alter the relative number of various Nups at the NPC through any of these mechanisms, leading to changes in the number of Pom152p-associated Nups at the pore. These changes would likely alter the stoichiometry of Nups at each NPC, resulting in altered function. Our observation that a deletion of *POM152* is synthetically lethal with *nup1Δ* while *pom152* alleles lacking glycosylation sites suppress *nup1Δ* temperature sensitivity suggests that a reduction in Pom152p glycosylation does not lead to a loss of functional Pom152p protein at the NPC. In addition, we observe that wild-type Pom152p and Pom152ΔGlyc protein are present at similar levels (Figure 6A), suggesting that Pom152ΔGlyc is not being rapidly degraded. Instead, changes in the glycosylation state of Pom152p may alter NPC assembly and/or structure. The transmembrane nucleoporins Pom152p and Ndc1p may assemble early in NPC biogenesis to generate a precursor structure on which NPCs are formed (MARELLI *et al.* 2001). Indeed, an Ndc1p allele that is defective in NPC assembly has recently been identified (LAU *et al.* 2004). Conditional alleles encoding the Pom152p-associated nucleoporin Nic96p result in a decrease in NPC number under growth at the nonpermissive temperature (GOMEZ-OSPINA *et al.* 2000). While changes in NPC structure and number have not been assessed in *pom152* mutant cells, the deletion of *pom152* does not alter the rate of diffusion through the NPC of several GFP-tagged reporter proteins (SHULGA *et al.* 2000), indicating that NPC structure and/or number is not altered significantly enough in the absence of Pom152p to affect passive transport through the nuclear pore.

The genetic screen reported here has identified up to 32 complementation groups that suppress the temperature sensitivity of a *nup1Δ* mutant. Since these mutants were generated by integrating a transposon insertion library into the *nup1Δ* starting strain, most of these mutations are presumably loss-of-function mutants created by the disruption of an open reading frame with the transposon. How might the generation of a second-site "deletion" result in the suppression of *nup1Δ* temperature sensitivity? One possibility is that the loss of Nup1p at the nuclear basket of the NPC slows the translocation of importin/cargo complexes through the NPC. The accumulation of these complexes within the nuclear basket may create a steric block within the NPC channel, resulting in the loss of translocation of some essential nucleophilic substrates across the NE. Suppression could then occur through the loss of some upstream step within the same pathway, preventing the importin/cargo complexes from ever entering the NPC and thus preventing an accumulation of importin/cargo within the NPC. Alternatively, suppression could be the result of a change in a second pathway that enhances translocation through the NPC, potentially via

a Nup1p-independent pathway. In either case, some of the suppressor mutations are likely not to be alleles of genes that encode direct constituents of a pathway that mediates translocation (*i.e.*, Nups or Kaps), but instead encode proteins that regulate the activity of factors directly involved in transport across the NPC.

Our results provide evidence that the glycosylation of NPC components influences NPC function, possibly by inhibiting a transport step upstream of Nup1p, enhancing a parallel import pathway that does not require Nup1p, or altering the structure of the NPC in such a way that cNLS import is enhanced in a *nup1Δ* mutant. The large number of spontaneous suppressors that we observed and the Tn-induced *bnps* that we have isolated support the likelihood that proteins other than nucleoporins and the relatively small number of soluble nuclear transport factors can influence NPC function and/or structure. A similarly large number of mutants were isolated in a genetic screen for mutants in NPC assembly and structure (RYAN and WENTE 2002). In this screen, EMS mutagenesis of cells expressing Nup-GFP reporters generated up to 87 *npa* (NPC assembly) complementation groups in which several Nups, including Pom152p, are mislocalized at elevated temperatures. While some of these *npa* mutants encode alleles of the Ran-GTPase and its regulators (RYAN *et al.* 2003), several mutants also encode factors important for ER function (RYAN and WENTE 2002), and most of the *npa* mutations remain unidentified. Each of the *sec* mutants identified in this screen produced aberrant ER structures, suggesting that abnormal proliferation of the ER (and thus of the NE) may result in aberrant NPC assembly. However, it remains possible that these mutants, either directly or indirectly, influence the efficiency of glycosylation in the ER and that the failure of Pom152p and other Nups to appropriately incorporate into the NPC is a consequence of changes in the glycosylation of Pom152p or other ER/NE components. The cloning of additional *bnp*'s, *npa*'s, and other mutations influencing NPC function will likely identify new signaling pathways and/or post-translational modifications that regulate NPC activity.

Here we have provided evidence that glycosylation is important for NPC function, at least in part through the post-translational modification of the luminal domain of Pom152p. Future work will examine whether changes in the glycosylation state of Pom152p influence transport kinetics through the NPC and/or alter the composition or assembly of NPCs. Since the two additional transmembrane nucleoporins in yeast, Pom34p and Ndc1p, also contain predicted glycosylation sites in their luminal domains, further investigation of their role in nuclear transport and assembly may also reveal insights into the regulation of NPC activity by glycosylation.

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