Regulation of mRNA Export by Nutritional Status in Fission Yeast

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ABSTRACT

We have isolated a mutation in nup184(nup184-1) that is synthetically lethal with the mRNA export defective rael-167 mutation in Schizosaccharomyces pombe. The consequence of the synthetic lethality is a defect in mRNA export. The predicted Nup184p is similar to Nup188p of Saccharomyces cerevisiae, and a Nup184p-GFP fusion localizes to the nuclear periphery in a punctate pattern. The Δnup184 null mutant is viable and also is synthetically lethal with rael-167. In a rael+ background, both the nup184-1 and Δnup184 mutations confer sensitivity to growth in nutrient-rich medium (YES) that is accompanied by nuclear poly(A)+ RNA accumulation. Removal of the cAMP-dependent protein kinase, Pka1p, relieved the growth and mRNA export defects of nup184 mutants when grown in nutrient-rich medium. The activation of Pka1p is necessary, but not sufficient, to cause the severe poly(A)+ RNA export defects when nup184 mutant cells are incubated in YES, suggesting nutritional status can also regulate poly(A)+ RNA export. Our results suggest that the regulation of poly(A)+ RNA export by Pka1p kinase appears to be indirect, via a translation-dependent step, but post-translationally in response to YES.

NUCLEOCYTOPLASMIC transport of proteins and RNA proceeds through the nuclear pore complex (NPC). These processes are saturable and energy dependent (Gerace 1995; Izaurralde and Mattaj 1995; Gorlich and Mattaj 1996; Koepf and Silvers 1996; Gorlich 1997; Nigg 1997). The details of nuclear protein import have been well described (reviewed in Gorlich 1997; Weis 1998) and form the basis for models of export of proteins and RNA from the nucleus (Gorlich and Mattaj 1996; Nigg 1997; Ullman et al. 1997; Izaurralde and Adam 1998; Ohno et al. 1998; Weis 1998). In nuclear protein import, proteins containing a nuclear localization signal bind to receptors that mediate their interaction with the NPC and their transport through the pore. These receptors, also known as importins or karyopherins, are members of a family of proteins that are similar to importin-β (Fornerod et al. 1997b; Gorlich et al. 1997; Ohno et al. 1998). Similarly, it is thought that the export of proteins also proceeds through interaction between a receptor (exportin) and a nuclear export signal (NES) on the cargo protein (Fornerod et al. 1997a; Fukuda et al. 1997; Neville et al. 1997; Ossareh-Nazari et al. 1997; Stade et al. 1997). Recently, Cmn1p, also known as Xpo1p, has been identified as the receptor required for export of proteins containing the leucine-rich NES (Fornerod et al. 1997a; Fukuda et al. 1997; Neville et al. 1997; Ossareh-Nazari et al. 1997; Stade et al. 1997). Because mRNA is exported through the nuclear pore as an mRNP particle (Visa et al. 1996; Danesholt 1997), the prevailing model is that carrier proteins in this mRNP contain NES signals that mediate interaction with an export receptor, which in turn directs the export of the mRNP particle through the NPC.

In Schizosaccharomyces pombe, the Rae1p is an important factor required for mRNA export. Temperature-sensitive rael mutants rapidly accumulate nuclear poly(A)+ RNA after a shift to restrictive conditions (Brown et al. 1995). We have isolated several mutations that are synthetically lethal with the rael-167 mutation. We have characterized one mutation in nup106 that encodes a homologue of the Saccharomyces cerevisiae Nic96p (Yoon et al. 1997). Nic96p is a major nonrepeat nucleoporin that interacts both genetically and physically with Nup188p, another nonrepeat nucleoporin (Nehr bass et al. 1996; Zabel et al. 1996). Nup188p also interacts with an integral membrane protein, Pom152p (Nehr bass et al. 1996). On the basis of the genetic and physical interaction of Nic96p with the repeat nucleoporin import complex, Nsp1p/Nup49p/Nup57p (Grandi et al. 1995), it is thought that Nic96p, along with Nup188p, links the structural framework of the NPC with the repeat nucleoporins that make up the transport machinery (Nehr bass et al. 1996; Zabel et al. 1996). Recently, it has been shown that the S. cerevisiae Rae1p homologue, Gle2p, binds to the GLFG-repeat nucleoporin, Nup116p (Baill er et al. 1998; Ho et al. 1998), and this binding is necessary for Rae1p localization to the nuclear pore (Baill er et al. 1998).

The export of RNA and proteins from the nucleus can be regulated. In S. cerevisiae and S. pombe, export of

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general mRNA is inhibited following heat shock and other forms of stress (Saavedra et al. 1996; Tani et al. 1996). However, heat-shock mRNAs are selectively exported, specifically the SSA4 mRNA, encoding the heat-inducible Hsp70p (Saavedra et al. 1996). The export of this class of mRNA requires Rip1p function, as well as other proteins required for bulk mRNA export (Gle1p), and several nucleoporins (Nup159p/Rat7p, Nup120p/Rat2p, and Nup145p/Rat10p) (Saavedra et al. 1997; Stutz et al. 1997). Recently, a transcriptional factor of S. pombe, Pap1p, activates genes in response to oxidative stress by localizing to the nucleus (Toone et al. 1998). The model is that Pap1p shuttles between the nucleus and cytoplasm and that its export is inhibited by stress via a mitogen-activated protein (MAP) kinase signal transduction pathway. Consistent with this hypothesis is the observation that loss of crm1 function, shown to be an importin-β-like export receptor (Forreod et al. 1997b; Fukuda et al. 1997; Neville et al. 1997; Ossareh-Nazari et al. 1997; Stade et al. 1997), also accumulates Pap1p in the nucleus at restrictive temperatures (Toone et al. 1998). It is not known whether Crm1p, another component of the export machinery, or Pap1p itself, is the target of the MAP kinase pathway.

Here we report the identification of a mutation in nup184, nup184-1 that is synthetically lethal with the temperature-sensitive mRNA export mutation rae1-17. Nup184p is similar to S. cerevisiae Nup188p (Nehrbass et al. 1996; Zabel et al. 1996), and a Δnup184 mutant is viable. However, both nup184-1 and Δnup184 mutations confer an mRNA export defect that is responsive to growth conditions. Whereas mild mRNA export defects were observed in synthetic minimal medium, there was a severe mRNA export defect when cells were grown in nutrient-rich media containing yeast extract. This effect on mRNA export that was uncovered in nup184 mutant cells appears to be mediated in part by a CAMP-dependent regulatory pathway and by a pathway that senses nutritional status.

**MATERIALS AND METHODS**

Strains and culture: Strains used in the study are listed in Table 1. The basic genetic and cell culture techniques used have been described (Moreno et al. 1991; Alfa et al. 1993). Appropriately supplemented Edinburgh minimal medium (EMM) was used to express genes from the nmt promoter (Maundrell 1993). The nmt promoter was repressed by the addition of 0.5 μM thiamine in EMM.

Separation of the s27 synthetic lethal mutation: Isolation of the SL27 synthetic lethal mutant has been described (Yoon et al. 1997). To determine whether the s27 mutation conferred a discernible phenotype in a rae1-1 background, SL27 was crossed with a Ura- Leu- strain and a random spore analysis was performed. Spores were plated onto EMM lacking leucine to select for those spores that retained the pREP81X-rae1-1 plasmid. The following three categories of colonies were obtained: (i) Colonies that were unable to grow in the presence of thiamine at 28° were judged to have both the rae1-167 and s27 mutations, (ii) colonies that were able to grow in the presence of thiamine at 28° but not at 36° were judged to have the rae1-167 mutation, and (iii) colonies that were able to grow in the presence of thiamine at 28° and 36° were judged to be rae1-1 and could carry either the wild-type or s27 alleles. Colonies carrying the s27 allele were identified by a backcross to the parental rae1-167/pREP81X-rae1-1 strain. Those colonies that produced spores with a synthetic lethal phenotype were judged to carry the s27 mutation. These s27 mutants grew normally at all temperatures (21° to 36°) on EMM, but grew very slowly when plated on nutrient-rich YES medium. To test whether this YES-sensitive (YES) phenotype was related to the synthetic lethal mutation, YES cells were sequentially crossed against a wild-type strain four times. In the final cross, a tetrad analysis was performed and the YES phenotype showed a 2:2 segregation. Six YES colonies from three tetrads were then crossed against the rae1-167 mutant bearing the pREP81X-rae1-1 plasmid. Random spore analysis revealed that all six YES colonies produced colonies that were unable to grow in the presence of thiamine at 28°.

**Identification of the s27 gene and the s27 mutation:** The cognate s27 gene was obtained by transformation of SL27U [same as SL27 except that rae1-1 is expressed from the pREP82X (Forberg 1993; Maundrell 1993) vector with uracl as a selectable marker] with a partial HindIII library cloned into pHW5 (Wright et al. 1986) and selection for Leu+ colonies that could grow at 28° in the presence of thiamine. Those transformants that received a plasmid carrying rae1-1 were identified by their ability to grow at 36° in the presence of thiamine. One colony was identified that did not carry a rae1-1 plasmid and allowed growth of SL27U in the presence of thiamine at a more restrictive temperature of 30°. We were unable to rescue the complementing plasmid. The complementing insert was isolated by PCR amplification using the XL PCR kit (Perkin-Elmer, Branchburg, NJ) with primers for the cDNA to select for those spores that retained the pREP81X-rae1-1 plasmid. Random spore analysis revealed that all six YES colonies produced colonies that were unable to grow in the presence of thiamine at 28°.

**Construction of the Δnup184::ura4 null mutant:** The Δnup184::ura4 null was constructed in the following manner. First, a HindIII site was created at the C terminus by PCR mutagenesis. The deletion was then constructed by ligation of the 5' SalI HindIII and the 3' HindIII-SpeI fragments into pBluescript. A 5.9-kb Scal fragment was cloned into pDW232 and the resulting plasmid, p184Sca, could complement SL27. The nup184 cDNA was isolated as described previously (Yoon et al. 1997). The pUZ184 plasmid was made by replacing the coding region of nup184 with the cDNA sequence. The cDNA and 5.9-kb Scal fragment were sequenced by SAIc (Frederick, MD).

**Construction of the Δnup184::ura4 null mutant:** The Δnup184::ura4 null was constructed in the following manner. First, a HindIII site was created at the C terminus by PCR mutagenesis. The deletion was then constructed by ligation of the 5' SalI HindIII and the 3' HindIII-SpeI fragments into pBluescript. A HindIII fragment carrying the ura4+ marker was then inserted into the HindIII site of the deletion. The SalI-SpeI fragment carrying the Δnup184::ura4 deletion was then transformed into the h+/h- diploid, SP286. Stable Ura+ transformants were screened by Southern blotting for the replacement of one of the nup184 genes. Several h+/h- colonies were identified (h+ converts spontaneously to h-, allowing sporation of the diploid), and dissection of 30 tetrads did not yield a viable Δnup184::ura4 spore in YES medium. Microscopic inspection of the spores that did not form colonies revealed that they were unable to germinate. Random spore analysis also did not yield any viable Δnup184::ura4 spores in EMM medium. The diploid was then transformed with a nup184+ plasmid with leucine as a selectable marker. This strain was sporulated and dissection of 24 tetrads yielded four viable spores when germinated on YES medium. Most of the tetrads gave rise to two large and two very small colonies. The two small colonies carried the Δnup184::ura4 null mutation. Other tetrads produced either three large colonies and one small colony or four large colonies. In each of these tetrads, the Δnup184::ura4
had segregated 2:2, with the large Ura \(^+\) colonies carrying the nup184 \(^+\) plasmid. When the small Ura \(^+\) colonies were plated on EMM, they were able to form large colonies, but like the nup184-1 mutant, the nup184::ura4 null was YES\(^+\) (data not shown).

**Construction of green fluorescent protein (GFP) fusions:** The pUZ184GFP plasmid was made by inserting a XbaI-GFP-BamHI fragment into a derivative of pUZ184, which had a XbaI site inserted at the C terminus. To integrate this construct a SacI-nup184-GFP-BamHI fragment was inserted into a derivative of pDW232 lacking the ARS sequence (pDW234). The resulting plasmid was integrated into the nup184 locus following linearization by KpnI and transformation into wild-type and rae1-167 cells. Stable Ura \(^+\) transformants were screened by Southern blotting for identification of the strain with a copy and transformation into wild-type digitized with a Polaroid Sprint Scan scanner and the images processed with the Image Pro Plus software. Electron microscopy: Fluorescent and electron microscopy: Fluorescence in situ hybridization for Poly(A)\(^+\) RNA was performed as previously described (Brown et al. 1995), using an oligo(dT)\(^50\)-labeled probe followed by a FITC-labeled antidigoxigenin Fab antibody. GFP fusion proteins were visualized either in live cells or following fixation for 5 min in 2% formaldehyde and 0.05% glutaraldehyde in PBS at room temperature. Cells were visualized using a Zeiss (Thornwood, NY) Axioshot microscope with \(\times 63\) and \(\times 100\) objectives and photographed with Kodak Ektachrome 200 slide film (Rochester, NY). The slides were digitized with a Polaroid Sprint Scan scanner and the images processed with the Image Pro Plus software.

### TABLE 1

**List of strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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</thead>
<tbody>
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<td>rae1-167</td>
<td>h(^-) leu1-32 ura4-D18 rae1-167</td>
<td>Yoon et al. (1997)</td>
</tr>
<tr>
<td>Wild type</td>
<td>h(^+) leu1-32 ura4-D18 rae1-167</td>
<td>Brown et al. (1995)</td>
</tr>
<tr>
<td>SL27</td>
<td>h(^-) leu1-32 ura4-D18 rae1-167 nup184-1 (pREP81X-rae1)</td>
<td>Yoon et al. (1997)</td>
</tr>
<tr>
<td>nup184-1</td>
<td>h(^-) leu1-32 ura4-D18 nup184-1</td>
<td>This study</td>
</tr>
<tr>
<td>Dnup184</td>
<td>h(^-) leu1-32 ura4-D18 Dnup184::ura4</td>
<td>This study</td>
</tr>
<tr>
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<td>C. Hoffman</td>
</tr>
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</tr>
<tr>
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<td>This study</td>
</tr>
<tr>
<td>SL27 Δpka1</td>
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<td>This study</td>
</tr>
<tr>
<td>Δcgs1</td>
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<td>M. McLeod</td>
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<td>This study</td>
</tr>
<tr>
<td>SP286</td>
<td>h(^+) h(^+) leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-210/ade6-216</td>
<td>D. Beach</td>
</tr>
</tbody>
</table>

**RESULTS**

**Isolation of synthetic lethal:** To identify genes involved in mRNA export, we have isolated mutations that are synthetically lethal when combined with the rae1-167 mutation (Yoon et al. 1997). Four different mutants were isolated that define one complementation group: SL8, SL22, SL27, and SL40. All of these mutants have the same growth and mRNA export phenotypes, and we have used SL27 for this study. The rae1-167 mutant grows normally at 28\(\circ\)C with normal poly(A)\(^+\) RNA distribution (Yoon et al. 1997), but it rapidly accumulates poly(A)\(^+\) RNA in the nucleus upon a shift to restrictive temperature (>30\(\circ\)). SL27 carries the rae1-167 and the synthetic lethal mutation, sl27, as well as a plasmid (pREP81X-rae1) that expresses the rae1 \(^+\) cDNA from the thiamine repressible nmt81 promoter. The sl27 mutation was separated and backcrossed to rae1-167 to confirm that the synthetic lethality was due to mutation of a single locus. Under permissive conditions when rae1 \(^+\) is expressed in the absence of thiamine, SL27 grows normally at 28\(\circ\). However, under synthetic lethal conditions when rae1 \(^+\)
protein is 184 kD and showed an overall 23% identity and 48% similarity when compared to Nup188p (Nehrbass et al. 1996; Zabel et al. 1996) of S. cerevisiae (GenBank accession no. AF055035). We named the gene nup184. The nup184 gene contained the sl27 mutation because a SacI-KpnI region was able to marker rescue the synthetic lethality of SL27. Sequence analysis of two independent PCR products using sl27 genomic DNA as template revealed a single mutation that introduces a termination codon at position 960 (Figure 1B). We named the sl27 mutation nup184-1.

nup184 is not essential but is required for growth on nutrient-rich media containing yeast extract: A null allele of nup184 was constructed in a h^+/h^+ diploid, SP286, by deletion from amino acid 143 to the end of the coding sequence and insertion of the ura4 gene (Figure 1B). From this diploid, we were able to isolate a haploid Δnup184::ura4 null mutant, indicating that it is not essential for growth (materials and methods). Like nup184-1, the Δnup184 null mutation is synthetically lethal when combined with the rae1-167 mutation, suggesting that nup184-1 is a loss-of-function allele. However, while both nup184-1 and Δnup184 mutants grew well on minimal EMM medium at all temperatures tested (18° to 36°), both grew extremely slowly on nutrient-rich medium containing yeast extract (YES; Figure 1C). This YES sensitivity (YES^s) cosegregates with both the nup184-1 and Δnup184 alleles.

Localization of Nup184p: To determine its localization, Nup184p was tagged at the C terminus with GFP. Expression of this fusion protein from pUZ184GFP was able to complement the synthetic lethality of SL27 (Figure 1A). The Nup184p-GFP fusion was then integrated at the nup184 locus (materials and methods). Fluorescent microscopic examination of the Nup184p-GFP fusion protein revealed its localization to the nuclear periphery in a punctate pattern that is similar to the staining for nuclear pores (Figure 2A). A similar construct fused to full-length Nup184p at the N terminus showed the same localization as the C-terminal fusion. However, the GFP-Nup184p-1p fusion, which has N-terminal 960 amino acids, no longer localized to the nuclear periphery (Figure 2A). Also, as with Nup188p (Nehrbass et al. 1996), deletion of the C-terminal 159 amino acids also prevented localization to the nuclear periphery (Figure 2A), and this truncation was unable to complement the synthetic lethality of SL27 (data not shown). On the basis of the sequence similarity to S. cerevisiae Nup188p (Nehrbass et al. 1996; Zabel et al. 1996) and its localization to the nuclear periphery, Nup184p is likely a nucleoporin.

Because the C-terminal truncations of Nup184p did not localize to the nuclear periphery, we tested the nuclear periphery localizations of Raelp in nup184-1 and Nup184p in rae1-167 mutants under permissive and nonpermissive conditions. Our results show no detectable differences in the nuclear periphery localizations.
Regulation of mRNA Export

Figure 2.—(A) Localization of Nup184p-GFP fusions. GFP was fused to full-length Nup184p at the C terminus and the construct was integrated at the nup184 locus. The GFP-Nup184-1p and the GFP-Nup184pΔC fusions are truncations of the C-terminal 670 residues and 159 residues, respectively, and both were expressed from a multicopy plasmid. Bar, 10 μm. (B) Thin-section electron micrographs of Δnup184 cells grown in EMM and transferred to YES medium for 24 hr are shown. Nuclear pores are indicated by arrowheads. Bar, 0.5 μm. (C) The effect of rae1-167 and nup184-1 mutations on the nuclear periphery localization of Nup184p-GFP and Rae1p-GFP fusions, respectively. The Nup184p-GFP was integrated at the nup184 locus in rae1-167 cells. These cells were grown at permissive temperature of 28°C and shifted to restrictive temperature of 36°C for 2 hr. The localization of Rae1p-GFP fusion expressed from a multicopy plasmid was analyzed in nup184-1 cells grown in EMM and shifted to YES for 12 hr at 28°C. Coincident 4',6-diamidino-2-phenylindole (DAPI) staining is shown in the bottom panels.

of Nup184p-GFP fusion in rae1-167 at 28°C or 36°C and the Rae1p-GFP fusion in nup184-1 mutant cells grown in EMM or in YES medium for 12 hr (Figure 2C). These results therefore suggest that the synthetic lethality of the rae1-167 nup184-1 double mutant and the YES sensitivity of nup184-1 is not due to the inability of Nup184p and Rae1p, respectively, to localize to the nuclear periphery.

Because in S. cerevisiae the combination of nup188 with either nic96 or pom152 mutations results in herniations of the nuclear envelope (Nehrbass et al. 1996; Zabel et al. 1996), the morphology of the nuclear envelope of SL27, Δnup184::ura4, and nup184-1 cells was examined by transmission electron microscopy. When the Δnup184 mutants were grown in YES medium, ~20% of the cells had a dilation of the nuclear envelope when compared to growth in EMM medium (Figure 2B). The distribution and number of pores does not appear to be significantly affected in these mutants. Similar results were seen for SL27 grown in the presence of thiamine and nup184-1 cells grown in YES medium (data not shown). Because the fraction of cells showing gross morphological defects was substantially less than the fraction of cells showing mRNA export defects in these mutants, the gross morphological defects conferred by these mutations cannot account for the mRNA export defects (see below).

mRNA export defects in SL27 and nup184 mutants:
Figure 3.—(A) Poly(A)^+ distribution in SL27, nup184-1, and Δnup184::ura4 mutants. Cells were grown to mid-log phase in appropriately supplemented EMM medium in the absence of thiamine (−B1) at 28°C. SL27 cells were then shifted to EMM medium containing thiamine (+B1) and grown for 24 hr. The nup184-1 and Δnup184::ura4 mutants were shifted to YES medium and grown for 12 hr at 28°C. Cells were fixed and the poly(A)^+ RNA was visualized by fluorescent in situ hybridization. Coincident DAPI staining is shown in the bottom panels. Bar, 10 μm. (B) Nuclear protein import analysis. Plasmids expressing the spMex67-NLS-GFP reporter protein expressed from the mex67 promoter were introduced into wild-type, SL27, Δnup184, and nup184-1 cells. SL27 cells were shifted to EMM medium containing thiamine (+B1) for 12 hr, and Δnup184 and nup184-1 cells were transferred to YES medium for 12 hr before in vivo nuclear import analysis was performed. spMex67-NLS-GFP reporter protein was visualized by fluorescent microscopy. Top panels (−energy) show diffusion of reporter protein in cells treated with 10 mM sodium azide and 10 mM deoxyglucose for 45 min. Bottom panels (+energy) show reimport of the reporter protein into the nucleus after cells were washed and resuspended in glucose-containing medium for 15 min to regenerate energy.

To determine whether the growth defects of the nup184 mutants were associated with mRNA export defects, the poly(A)^+ RNA distribution was examined in SL27, nup184-1, and Δnup184 mutants grown under restrictive conditions. When SL27 (rae1-167 nup184-1/pREP81X-rae1^-) was grown in the absence of thiamine, distribution of the poly(A)^+ RNA in the cells was normal (Figure 3A), but within 12 hr after the addition of thiamine and repression of rae1^- expression, ~90% of the cells showed strong nuclear accumulation of poly(A)^+ RNA. Under similar conditions, the poly(A)^+ distribution of RNA in rae1-167 was normal (Figure 3A; Yoon et al. 1997). Therefore, the synthetic lethality is associated with a defect in the export of mRNA from the nucleus. When the nup184-1 mutant was grown in EMM, ~5% of the cells had nuclear accumulation of poly(A)^+ RNA. Within an hour after a shift from EMM medium to nutrient-rich YES medium, nuclear accumulation of poly(A)^+ RNA was detected in ~25% of the cells (data not shown). As incubation continued, both the fraction of cells and the extent of nuclear accumulation increased. By 12 hr, 75% of the nup184-1 cells had strong nuclear accumulation of poly(A)^+ RNA (Figure 3A). Additionally, the Δnup184 cells showed a stronger mRNA export defect when grown in EMM where 50% of the cells had nuclear poly(A)^+ RNA accumulation. Incubation of Δnup184 cells in YES for 12 hr increased the strength and extent of nuclear poly(A)^+ RNA accumulation to a level similar to that seen for nup184-1 cells grown in YES (Figure 3A). Taken together, these results suggest that Nup184p has a role in mRNA export from the nucleus.
To determine whether another NPC function is affected in nup184 mutants, nuclear protein import of a SV40-NLS-GFP-LacZ reporter was examined (data not shown). In wild-type cells, in SL27 grown in the presence and absence of thiamine, as well as in Δnup184 and nup184-1 cells grown in EMM and YES medium, this reporter was localized exclusively to the nucleus with no reporter detected in the cytoplasm. As a control, the ptr3-1 mutant, which has a defect in protein import (Azad et al. 1997), accumulated a significant amount of the reporter protein in the cytoplasm within 3 hr after a shift to restrictive temperature. Because poly(A)$^+$ RNA export is inhibited in SL27 and nup184-1 mutants under restrictive conditions, we cannot exclude the possibility that the nuclear reporter protein is no longer synthesized under restrictive conditions due to cytoplasmic depletion of poly(A)$^+$ RNA. We also used a recently developed method for monitoring protein import that is not dependent on continued mRNA synthesis and export (Shulga et al. 1996). A spMex67-NLS-GFP fusion was expressed from a multicopy plasmid in wild type, SL27 grown in absence and presence of thiamine for 12 hr, and nup184-1 cells grown in EMM and in YES medium for 12 hr. This reporter localized predominantly in the nucleus (data not shown). However, when these cells were depleted for energy by treatment in glucose-free medium containing sodium azide and deoxyglucose, the low-molecular-weight reporter protein diffused into the cytoplasm (Figure 3B). After 45 min the cells were washed and returned to glucose-containing medium to restore energy. This allowed rapid reimport of the spMex67-NLS-GFP reporter in all cells within 15 min (Figure 3B). These results suggest that at least some of the transport functions of the NPC remain intact, and the mRNA export defect is not the result of the loss of pore function in general.

Sensitivity to growth on YES is responsive to a cAMP-dependent pathway: To understand the basis for the YES sensitivity of the nup184 mutants, we isolated a multicopy suppressor of the YES sensitivity of nup184-1. Sequence analysis of a clone, pKYR, revealed that it carried a gene, cgs2, encoding a cAMP-phosphodiesterase (Devoti et al. 1991). This raised the possibility that mRNA export is regulated by a cAMP-dependent pathway, and this regulation is revealed by the nup184 mutants. If activation of the cAMP-dependent pathway is necessary for the mRNA export defect, then inactivation of the pathway should allow growth of nup184-1 and suppress the mRNA export defect on YES. Therefore, nup184-1 was crossed with Δpka1, defective for the cAMP-dependent protein kinase in S. pombe, Pka1p (Yu et al. 1994). Indeed, removal of Pka1p relieved the growth defect and poly(A)$^+$ RNA export defect of the nup184-1 mutant when grown in YES (Figure 4, A and B). Moreover, inactivation of the cAMP-dependent pathway in SL27 suppressed the growth defect (Figure 4A) and mRNA defects of the synthetic lethality (Figure 4B). Because nup184-1 has partial activity (the Δnup184 mutant has a stronger mRNA export defect when grown on EMM; Figure 3A), it was possible that Δpka1 increased the activity of Nup184p, and this was responsible for the suppression. However, Δpka1 was also able to suppress the growth defect of Δnup184 in YES (Figure 4A) and rescue the poly(A)$^+$ RNA export defect of Δnup184 in EMM and in YES medium (compare Figure 4B to Figure 3A), suggesting that the Pka1p kinase pathway modulates a function other than Nup184p. Interestingly, there were some residual poly(A)$^+$ RNA export defects when Δpka1 nup184 mutants were grown in YES, leaving open the possibility that nup184 function could be regulated in part by nutritional status.

To test whether activation of the Pka1p kinase was sufficient to block mRNA export in nup184-1 cells, a Δcgs1 null mutation, removing the negative regulatory subunit of Pka1p (Devoti et al. 1991), was introduced into nup184-1 cells. Indeed, the poly(A)$^+$ RNA export defects of the Δcgs1 nup184-1 mutant were significantly higher in EMM medium when compared to individual mutants grown under similar conditions (Figure 4B). However, the poly(A)$^+$ RNA export defect of the Δcgs1 nup184-1 mutant in EMM is not as severe as in YES (Figure 4B). Interestingly, the Δcgs1 mutation itself conferred a modest nuclear poly(A)$^+$ RNA accumulation in nearly every cell (Figure 4B), suggesting that the cAMP pathway can downregulate mRNA export even in a nup184$^+$ background. Similar results were obtained when the Pka1p kinase pathway was activated by exogenous addition of 10 mm cAMP (8-bromo-cAMP) to wild-type cells grown in EMM (data not shown). However, addition of cAMP to wild-type cells, similar to Δcgs1 mutant, did not increase the poly(A)$^+$ RNA accumulation in the nucleus following shift into YES medium. Taking these results together, we conclude that the cAMP-dependent signal transduction pathway is necessary but not sufficient for the amount of export defect seen in nup184 mutants grown in nutrient-rich medium. Therefore, there is another pathway that senses nutritional status also contributing to the downregulation of mRNA export.

The mRNA export defect of nup184-1 in nutrient-rich medium is a post-translational event: Because cAMP levels and nutritional status regulate gene expression, the possibility exists that the mRNA export defect seen in nup184-1 is a result of lowered expression of a labile mRNA export factor that is required in the absence of cAMP. Because cAMP is regulated by a cAMP-dependent pathway, and this pathway is necessary for the mRNA export defect, then inactivation of the pathway allows growth of nup184-1 and suppresses the mRNA export defect, as was shown in Figure 4. Therefore, the mRNA export factor in nup184-1 is labile with respect to cAMP regulation.
mulation following the addition of cAMP to nup184-1 cells grown in EMM (Figure 5). Thus, the regulation by the cAMP-dependent Pka1p kinase is a translation-dependent event. This result and that of the Δpka1 null suppression of poly(A)+ RNA export defect in Δnup184 mutant in EMM and in YES medium suggest that Pka1p kinase activity is required for the expression of a factor necessary for the inhibition of mRNA export. While we cannot rule out nutritional regulation of the stability of an mRNA export factor, we favor a hypothesis in which a nutritional signal transduction pathway directly or indirectly regulates nucleocytoplastic trafficking by mod-
ifying the transport machinery. We conclude from our results that activation of the Pka1p pathway is necessary to negatively regulate poly(A)+ RNA export defect, but is not sufficient to cause the severe poly(A)+ RNA export defects seen in nup184-1 cells grown in nutrient-rich medium.

**DISCUSSION**

In this study, we have isolated a mutation in the nup184 gene, nup184-1, that truncates the C-terminal third of the protein. Both the nup184-1 and the viable
Δnup184 null mutations are synthetically lethal with the mRNA export defective rael-167 mutation (Yoon et al. 1997), and the consequence of this synthetic lethality is the nuclear accumulation of poly(A)⁺ RNA. Interestingly, both the Δnup184 and nup184-1 mutants are sensitive to growth in nutrient-rich medium containing yeast extract, and this sensitivity is also accompanied by an mRNA export defect. The sensitivity to growth of nup184 mutants in nutrient-rich conditions requires the Pka1p kinase, the S. pombe cAMP-dependent protein kinase, and a signal pathway sensitive to the nutritional status of the cell.

Nup184p is similar to S. cerevisiae Nup188p (Nehrbass et al. 1996; Zabel et al. 1996), one of several abundant nonrepeat nucleoporins that constitute a significant percentage of the mass of the NPC (Nehrbass et al. 1996). Immunoelectron microscopy has localized Nup188p to the core of the NPC with it being either a component of the spokes or the central transporter (Nehrbass et al. 1996). It genetically and physically interacts with Pom152p (Nehrbass et al. 1996), which is an integral membrane protein, and with another abundant nonrepeat nucleoporin, Nic96p (Nehrbass et al. 1996; Zabel et al. 1996). In S. cerevisiae, combination of nic96 (the homologue of S. pombe nip106) and nup188 mutations is synthetically lethal and the proteins physically interact (Nehrbass et al. 1996; Zabel et al. 1996). In S. pombe, a combination of Δnip106 and Δnup184 is not synthetically lethal (data not shown). The synthetic lethality between Nup188 and Nic96 in S. cerevisiae may have more to do with their roles in NPC biogenesis than in transport function. It has also been proposed that S. cerevisiae Nup188p functions by linking the structural components of the nuclear pore with the repeat containing nucleoporins that are thought to be part of the export machinery (Nehrbass et al. 1996; Zabel et al. 1996). Also, in S. cerevisiae the deletions of nup188 and a ts mutant ps4 confer severe morphological defects of the nuclear envelope with herniation without conferring any detectable nucleocytoplasmic defects under restrictive conditions. Examination of the nuclear envelope in nup184-1, Δnup184, and rael-167 nup184-1 cells grown under restrictive conditions revealed that in a portion of the cells there was swelling of the nuclear envelope (20%), though no herniations were seen. Moreover, because nuclear import of the reporter protein was unaffected in these mutants under restrictive conditions, the mRNA export defect does not appear to be due to complete inactivation of pore function. Following this hypothesis, loss of Nup184p could affect the functioning of the mRNA export machinery without completely inactivating it and at the same time could render mRNA export more susceptible to regulation by nutritional status.

The regulation by Pka1 kinase appears to be indirect since increased nuclear poly(A)⁺ RNA accumulation by addition of exogenous cAMP to nup184-1 was blocked when protein synthesis was inhibited. However, the regulation of poly(A)⁺ RNA export in response to nutritional status is post-translational, since inhibition of protein synthesis did not affect nuclear poly(A)⁺ RNA accumulation in nup184-1 cells following a shift to nutrient-rich medium. From these observations, we propose a model where cAMP-dependent Pka1p kinase is required for the expression of a factor that can negatively regulate mRNA export. The nutritional signal can function either through the Pka1p-dependent inhibitor or independently (Figure 6). However, both pathways of regulation are necessary to inhibit poly(A)⁺ RNA export in nup184 mutant cells when grown in nutrient-rich medium.

Activation of the Pka1 kinase pathway either by addition of cAMP to wild-type cells or removal of Cgs1p shows a modest nuclear accumulation of poly(A)⁺ RNA. Subsequent transfer of these cells to nutrient-rich medium does not further inhibit poly(A)⁺ RNA export when Nup184p is functional. Therefore, it is likely that Nup184p, either directly or indirectly, is involved in the regulation of poly(A)⁺ RNA export in response to the nutritional status. We considered two simple possibilities for the uncovering of mRNA export regulation by loss of nup184 function in response to the nutritional signals. One possibility is that loss of Nup184p in a complex could affect the regulation and function of proteins that...
negatively regulate poly(A)^+ RNA export in response to these signal transduction pathways. This could include the inability to regulate the nucleocytoplasmic localization of factors that can inhibit poly(A)^+ RNA export. Because the Pka1p kinase pathway is necessary to inhibit mRNA export, the ability of Δpka1 to suppress the poly(A)^+ RNA export defects seen in Δnup184 mutant cells in EMM or YES medium is consistent with this hypothesis. Thus, in wild-type cells, the export of a subset of transcripts is inhibited when cells are grown in YES medium. However, the capacity to export bulk poly(A)^+ RNA is not compromised, because the Nup184p-containing complex can regulate either directly or indirectly the function of the regulator(s) in response to the signals. A second possibility is that the removal of Nup184p reduces, but does not abolish, mRNA export. Normally, when Nup184p is present, inhibition of mRNA export by the nutritional status is either mild or specific for a subset of substrates. Thus, no bulk mRNA export defects are seen upon shifting from EMM to YES medium. However, removal of Nup184p affects the functioning of the mRNA export machinery so as to make it very sensitive to regulation by nutritional status. At this time we are unable to rule out either model.

In S. cerevisiae, the nup1 mutants, which has a temperature-sensitive Ran-GAP, rapidly accumulates nuclear poly(A)^+ RNA upon a shift to restrictive temperature (Hutchison et al. 1969; Shiokawa and Pogo 1974; Amberg et al. 1992; Forrester et al. 1992). Its growth defect can be partially relieved by growth on nonglucose carbon sources and also by mutations in REG1 (Tung and Hopper 1995). Reg1p is a regulatory subunit that targets protein phosphatase 1 to its transcription factor substrates (Tu and Carlson 1995). Interestingly, like the suppression of the nutrient-rich sensitivity of nup184-1 by multicopy cgs2, nup1-1 is suppressed by multicopy PDE2 (Tung and Hopper 1995), encoding the cAMP-phosphodiesterase of S. cerevisiae related to Cgs2p (Devoti et al. 1991). Moreover, the suppression by reg1 mutations is abolished by constitutive activation of cAMP-dependent protein kinase activity by a Δbcyl mutation (Tung and Hopper 1995), lacking the regulatory subunit of cAMP protein kinase. Recently, PDE2 was isolated as a suppressor of gsp1, the S. cerevisiae homologue of the Ran GTPase, an essential factor in nuclear cytoplasmic trafficking (Oki et al. 1998). These observations, together with regulation of mRNA export by the cAMP-dependent signal transduction pathway reported here, raise the possibility of common factors in the regulation of the nucleocytoplasmic transport machinery in these two yeasts. Identification of these targets should provide valuable information in understanding how transport through the NPC can be regulated.

In S. pombe, nutritional status regulates growth in part by regulating cell cycle G2/M transition. This is revealed in spa1 mutants, also known as sty1, that have an extended G2 delay when grown in minimal medium. Spa1p is a MAP kinase that is activated by the Wis1p MAP kinase kinase (MAPKK) in response to osmotic stress and nutrient limitation. This G2 delay in spa1^-1 cells is relieved by growth in nutrient-rich medium containing yeast extract (Shiozaki and Russell 1995). It is interesting to note that temperature-sensitive rae1-1 mutant cells undergo a G2/M cell cycle arrest that is independent of the mRNA export defect (Whalen et al. 1997). Moreover, mutations in the rae1 homologue of Aspergillus nidulans, sonA, affect the localization of cyclin B in a nimA1 mutant (Wu et al. 1998). The genetic interaction between rae1 and nup184, along with the nutritional effects on mRNA export in nup184 mutant cells, raises the interesting possibility that these processes are interconnected. We are investigating whether the Wis1p-Spa1p MAP kinase cascade participates in regulation of mRNA export in response to nutritional status.

In S. cerevisiae and S. pombe, bulk mRNA export is inhibited during a heat shock (Saavedra et al. 1996; Tani et al. 1996), and in S. cerevisiae heat-shock RNA has been shown to be selectively exported (Saavedra et al. 1996). One model for this regulation is that heat shock mRNA and bulk mRNA compete for access to the export machinery (Saavedra et al. 1997; Stutz et al.)
al. 1997). Under heat-shock conditions, there is preferential export of the more efficient heat-shock mRNA substrates. Similarly, nutritional status regulates the expression of genes to provide the proper balance between the expression of genes involved in biosynthesis and those involved in macromolecular synthesis. In nutrient-rich conditions, there is a greater demand for mRNA encoding macromolecular synthesis proteins. Conceivably, one level of regulation could be controlling the relative export efficiencies of biosynthetic and macromolecular synthesis mRNAs. The nup184 mutations could uncover this regulation by diminishing the overall function of the export machinery.

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