Abstract. To extend our understanding of the mechanism by which the nuclear pore complex (NPC) mediates macromolecular transport across the nuclear envelope we have focused on defining the composition and molecular organization of the yeast NPC. Peptide sequence analysis of a polypeptide with a Mr of ~100,000 present in a highly enriched yeast NPC fraction identified a novel yeast nucleoporin we term Nup120p. Nup120p corresponds to the open reading frame (ORF) YKL057c identified by the yeast genome sequencing project. The ORF predicts a protein with a calculated molecular mass of 120.5 kD containing two leucine zipper motifs, a short coiled-coil region and limited primary sequence similarity to Nup133p. Nup120p was localized to the NPC using a protein A-tagged chimera in situ by indirect immunofluorescence microscopy. Deletion of the NUP120 gene caused clustering of NPCs at one side of the nuclear envelope, moderate nucleolar fragmentation and slower cell growth. Transfer of nup120A cells to 37°C resulted in the nuclear accumulation of poly(A)+ mRNA, extensive fragmentation of the nucleolus, spindle defects, and cell death.

Nuclear pore complexes (NPCs) span circular openings in the nuclear envelope (NE) where they govern bidirectional macromolecular traffic between the nucleus and cytoplasm. The NPC is a huge (~125 MD in vertebrates and ~65 MD in yeast) octagonally symmetric cylindrical structure. Investigation of the NPC structure by electron microscopy and high resolution image reconstruction has revealed a complex subunit organization. Four coaxial rings lie parallel to the nuclear envelope and are joined by eight spokes: two morphologically symmetric cytoplasmic and nuclear rings flank the core structure of the NPC at the nuclear and cytoplasmic faces; an outer spoke ring lies within the lumen of the nuclear envelope and a nuclear ring surrounds a central transporter. Eight cytoplasmic filaments and particles emanate from the cytoplasmic ring, and eight filaments attached to the nucleoplasmic ring extend to a distal basket ring and comprise the nuclear basket (Unwin and Milligan, 1982; Jarnik and Aebi, 1991; Goldberg et al., 1992; Hinshaw et al., 1992; Akey and Radermacher, 1993; Ris and Malecki, 1993). The NPC has been estimated to contain at least 40 nucleoporins (nups) and pore membrane proteins (poms) some of which have been identified and molecularly characterized.
In an effort to examine the precise roles of nucleoporins in the subunit organization of the NPC and macromolecular transport processes we have extended our characterization of yeast nucleoporins to a novel essential nucleoporin, termed Nup120p. Nup120p was isolated from a highly enriched yeast NPC fraction and identified by direct microsequencing. The protein shows limited primary sequence similarity to Nup35p (Doye et al., 1994; Pemberton et al., 1995; Li et al., 1995) and contains two leucine zippers and a third potential putative coiled-coil domain. Deletion of the NUP120 gene causes pores to cluster at one side of the nuclear envelope and, at elevated growth temperatures, poly(A)+ mRNA accumulation in the nucleus, extensive nucleolar fragmentation, spindle defects, and cell death.

Materials and Methods

Yeast Strains and Media

All yeast strains used in this study were derived from the strain DFD (Mata/Mata ura3-32 his3-32 Δhis2Δhis3 Δ200 trp1-1/Δtrp1-1 leu2-3,112 lys2-801 lys2-801; Aitchison et al., 1995). NPI20-25-3 (Mata ura3-32 his3-32 Δ200 trp1-1 leu2-3,112 lys2-801 nup120-25) was a haploid segregant of the heterozygous diploid NP120-25 generated by integration of the transformation cassette into the URA3 gene as described by Rothstein (1991). The following primers and the plasmid pProtA/HU were designed to generate the URA3 gene by direct integration of a PCR product encoding the IgG binding domains of protein A as previously described (Aitchison et al., 1995). The following primers and the plasmid pProtA/HU were designed to generate a URA3 gene which encodes the IgG binding domains of protein A at the 3′ end of NUP120 followed by the HIS3 and URA3 genes:

```plaintext
sense primer: 5'-TAATITAGATITAAAGTGCAATCTITFTATTTAAAAAC-
antisense primer: 5'-TACGATCAATGGATTCTAAACGGAAGTAAAGTGGTACT-
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Identification of Nup120p

Proteins comprising a highly enriched yeast nuclear pore complex fraction (~10 mg of total protein) were separated by ion exchange chromatography, reverse phase HPLC, and SDS-PAGE as described (Rout and Blobel, 1993; Wozniak et al., 1994). A protein of this fraction with a Mr of 3,112/leu2-3,112 lys2-801/lys2-801; Wozniak et al., 1994). A protein of this fraction with a Mr of 30°C unless otherwise stated. Procedures for yeast manipulation were conducted as described by Sherman et al. (1986). Yeast were transformed by electroporation (De郎erre et al., 1989).

Gene disruption and Protein A Tagging of NUP120

The NUP120 gene was replaced in the DFD cells by direct integration of the URA3 gene using the procedure of Rothstein (1991). The URA3 gene with flanking NUP120 DNA sequence was generated by PCR using the following primers and the plasmid pURAGal (Aitchison et al., 1995) as a template:

```plaintext
sense primer: 5'-CATAAAGGAGTTTCTAACAGAAGGTCGAATTCACTCAG-
antisense primer: 5'-AGTAACACATTTACTCCTGTTTAGAATCTCATTAGCTG-
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Examination of Synthetic Lethality

Double null mutants of nup120 and nup170, nup188, nup133, or pom152 were produced by crossing NPI20-25-3 cells with each of the following: PM152-75 (Mata ade2 ade3 his3 trp1 leu2 can1 pom152::HIS3), NPI170-11.1 (Mata ade2-1 ura3-3 his3-11,15 trp1-1 leu2-3,112 can1-100

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**Results**

**Identification of Nup120p**

The isolation of a fraction of yeast highly enriched intact NPCs (Rout and Blobel, 1993) has facilitated the identification of a growing number of nucleoporins. We have extended this biochemical approach to the identification and characterization of a novel yeast nucleoporin, termed Nup120p. Separation of the proteins of the highly enriched NPC fraction by anion exchange chromatography followed by HPLC and SDS-PAGE allowed us to identify a polypeptide with an estimated $M_r$ of ~100,000 which co-enriched with NPCs during the isolation (Fig. 1 A). Two peptide sequences derived from this polypeptide matched exactly with the ORF YKL057c on the left arm of chromosome XI sequenced in the yeast genome sequencing project (Fig. 1 B). Based on its co-isolation with NPCs and in situ evidence described below, we have renamed this ORF, NUP120. A search of the GenBank/EMBL/DDBJ and Swiss-Prot databases using the FASTA program failed to identify any extended regions of sequence similarities with other proteins in the database. Interestingly, however, a short region (amino acids 98-420) of Nup133p (Doye et al., 1994; Li et al., 1995) was identified which showed 20% identity and 62% similarity to amino acids 434-763 of Nup120p (Fig. 1 C). This region of homology corresponds to the amino-terminal portion of Nup133p which has been previously shown to be required in Nup133p for the spatial localization of NPCs (Doye et al., 1994; Wente et al., 1993) and subcellular fractionation of these cells showed coenrichment of Nup120p-protein A with the nuclear envelope (data not shown).

**Deletion of NUP120 causes Temperature Sensitivity**

The gene encoding Nup120p was disrupted in the diploid yeast strain DF5 by integrative transformation of the URA3 gene (Fig. 3 A; see Materials and Methods). The heterozygous diploid $nup120::URA3$ strain was sporulated and tetrads were dissected. Four viable spores were recovered from most tetrads; however, the segregants carrying the disruption ($nup120::URA3$) grew much slower and were noticeably larger than the cells with the wild-type NUP120 gene, and failed to grow when plated at 37°C (Fig. 3 B).

To examine this growth defect further, the growth rates and survival of $nup120\Delta$ (N1P120-25-3) cells were monitored over a 24 h period (Fig. 3 C). At 23°C $nup120\Delta$ cells grew with a generation time of approximately 4–5 h compared to approximately 2 h for wild-type cells. After transfer to 37°C the number of $nup120\Delta$ cells doubled over the first 10–15 h. At both temperatures $nup120\Delta$ cells appeared larger by light microscopy. The viability of the cells was monitored by removing aliquots from the liquid cultures and plating at 23°C (Fig. 3 D). $nup120\Delta$ cells rapidly lost viability after approximately 10 h at 37°C, and after 24 h, less than 1% of the cells were able to form colonies. The loss of viability of $nup120\Delta$ cells at 37°C coincides with the strain’s generation time, suggesting that cells can undergo only 1–2 divisions before they die at the restrictive temperature.

$nup120\Delta$ ($nup120::URA3$) cells were crossed with strains harboring deletions of the genes encoding other NPC proteins in order to examine possible genetic interactions. Crosses were made with pom152Δ (PM152-75: pom152:: HIS3), nup170Δ (N1P170-11.1: nup170::HIS3) (Aitchison et al., 1995), nup188Δ (N1P188-2.4; nup188::HIS3; Nehrbass, U. S. Maguire, M. P. Rout, G. Blobel, and R. W. Wozniak, manuscript submitted) and nup133Δ (3-4; nup133::HIS3; Pemberton et al., 1995). Approximately twenty tetrads of each cross were dissected and examined for segregation of markers and viability. In no case could viable haploid strains carrying both markers (His+, Ura+) be recovered and each

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of the predicted double mutants failed to form colonies at 23°C. These results suggest synthetic lethality of the NUP120 deletions with the four strains above.

**Nuclear Pore Complexes Cluster in nup120Δ Cells**

The characteristic punctate pattern of NPCs revealed by immunofluorescence microscopy using antibodies directed against NPC components represents the distribution of NPCs throughout the nuclear envelope. While this pattern is also observed with antibodies directed against the NPC marker protein Nup159p (Kraemer et al., 1995) in wild type cells, the NPCs of nup120Δ cells stained for this same marker were found clustered as a cap on one side of the nuclear envelope (Fig. 4). This was also the case for anti-Nsp1p antibodies (see below) and several other antibodies against NPC proteins (data not shown). The size and staining intensities of these capped structures varied markedly; indeed, some cells seemed to contain little, if any, staining material. Although most of the stained material in each cell was contained within the large cap, it was not infrequent for the remainder to be localized to one or more considerably smaller clusters located elsewhere on the nuclear envelope. This altered distribution was observed at both the semi-permissive temperature of 23°C and after the shift to 37°C, although after 6 h at the restrictive temperature the caps were often smaller and the cells contained less staining material overall (data not shown). Sim-
Figure 2. Immunofluorescent localization of Nup120p-protein A. Nup120 was tagged by an in-frame insertion of the IgG binding domains of protein A into the genomic NUP120 gene generating a COOH-terminal fusion (A). The chimera was visualized in formaldehyde fixed yeast cells by affinity purified rabbit IgG followed by Cy3-labeled donkey anti-rabbit IgG revealing punctate nuclear rim staining characteristic of NPC-localization (B). The nuclear DNA was visualized by coincident staining with DAPI. Bar, 5 μm.

Similar staining has been observed with deletion of NUP133 (Li et al., 1995; Doye et al., 1995; Pemberton et al., 1995), a temperature sensitive mutation of NUP159 (Gorsch et al., 1995) and an amino terminal deletion of NUP145 (Wente and Blobel, 1994).

To establish if the clustered NPCs were distributed randomly within the nuclear envelope, double immunofluorescent labeling was performed using mouse monoclonal antibodies directed against an abundant nucleolar protein Noplp (Aris and Blobel, 1988) and rabbit polyclonal antibodies against Nsp1p (Nehrbass et al., 1990) (Fig. 5 A). The position of the NPC cap in each cell is marked in green,

Figure 3. Deletion of NUP120 results in temperature sensitivity, affecting growth rate and viability of nup120A cells. Deletion and disruption of the NUP120 gene was accomplished by replacement of the NUP120 ORF with the URA3 selectable marker in the diploid strain DF5 (A). Heterozygous diploids containing a wild-type and a disrupted copy of NUP120 were sporulated and tetrads dissected on YPD plates. While all four spores could be recovered, haploid cells carrying a disrupted copy of NUP120 failed to grow at 37°C (B). nup120Δ cells (nup120::URA3) and haploid DF5 cells (WT) were grown in liquid medium at 23° and 37°C and were then counted at the indicated times (C). The number of viable cells was determined by plating aliquots of the cultures onto YPD and counting the colonies after 3 d at 23°C. nup120Δ cells failed to survive after one to two divisions at 37°C (D).
Figure 4. Nuclear pores cluster in nup120Δ cells. Examination of the NPCs of nup120Δ (nup120::URA3) and wild-type (WT) cells by immunofluorescence directed against the NPC marker protein Nup159p. NPCs are distributed throughout the nuclear envelope in wild-type cells whereas NPCs are clustered at one side of the nuclear envelope in nup120Δ cells at both the semi-permissive temperature 23°C and after the shift to 37°C (not shown). NPCs were visualized using the monoclonal antibody to Nup159p MAb165C10 (Kraemer et al., 1995) followed by Cy3-labeled donkey anti-mouse IgG. The nuclear DNA was visualized by coincident staining with DAPI. Bar, 5 μm.

whilst the nucleolus is red. Very few cells have coincident staining (yellow). These results indicate that the two structures generally occupy different regions of the nuclear envelope. In order to quantify this spatial arrangement, the orientation of the NPC cap was scored relative to the nucleolus by dividing the nuclear periphery into three areas: (a) opposite (~135°-225°); (b) adjacent (~45°-135° or ~225°-315°); and (c) coincident (~315°-45°) relative to the nucleolus (center at 0°) and analyzing overlaid fluorescent images. Since cells were oriented randomly, only cells where the crescent shape of the nucleolus (Aris and Blobel, 1988) could be clearly visualized were examined for the relative position of the NPC cap structure (Yang et al., 1989). In approximately 85% of the cells the NPC cap structure was oriented opposite to the nucleolus, whereas less than 3% of the cells showed coincident nucleolar and NPC staining (Fig. 5 B). Even this apparent coincidence may be explained by the orientation of the nucleus, such that the nucleolus lies directly above or below (though still separate from) the NPC cluster. These results suggest that the orientation of the clustered NPCs is not random and may reflect a higher order structure within the nucleus.

Effect of NUP120 Deletion on Nuclear Markers

In order to determine whether the deletion of NUP120 affected nuclear structures other than the nuclear envelope, we compared the distribution of different nuclear markers by immunofluorescence microscopy in nup120Δ cells and wild-type cells at various times after a shift to the restrictive temperature. The most striking observation was that of a progressive disintegration of nucleolar morphology after the temperature shift in nup120Δ cells, as visualized with a monoclonal antibody against Nop1p (Aris and Blobel, 1988). In wild-type cells, nucleoli stained with this antibody appear as crescent-shaped structures, faintly lobulated, and with their convex surface tightly apposed to the inner face of the nuclear envelope (Fig. 6 A). This morphology was found to be similar at all time points after the temperature shift. In contrast, the nucleoli of nup120Δ cells were slightly abnormal even at the semi-permissive temperature (Fig. 6 A).

Approximately 20% of the cells showed some signs of nucleolar fragmentation, which was usually manifested as one or two small, spherical patches of staining near a nucleolus of approximately normal size and appearance. However, after 1 h, there was a dramatic increase in the proportion of fragmented nucleoli. Over 70% of the cells contained the spherical patches, which were larger and could number as many as five per cell. Nucleoli, if distinguishable, appeared to be more diffuse and less sharply associated with the nuclear envelope. This phenotype had become slightly more pronounced by 3 h (Fig. 6 A). By this time point, most of the nucleoli were smaller, and some were difficult to locate at all. The overall intensity of staining was lower than for comparable wild-type cells, or mutant cells at the semi-permissive temperature. After 6 h, a normal nucleolus could be found in only a few cells, the rest carrying numerous spherical patches of varying size and intensity (data not shown). Nucleolar dispersal made it impossible to correlate the position of the capped NPCs with the nucleolus at the restrictive temperatures. This nucleolar fragmentation was also observed by thin-section electron microscopy (Fig. 6 B). At the semi-permissive temperature, each nucleolus appeared as a single denser region of chromatin appressed to the nuclear envelope and opposite the clustered NPCs (Fig. 6 B, arrows). After 3 h at the restrictive temperature, the nuclei of many cells

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contained several denser regions of chromatin, most being circular in section and detached from the nuclear envelope, spread throughout the nucleoplasm (Fig. 6 B, arrows).

The spindle was another nuclear marker whose structure became markedly altered as a result of the temperature shift. Measurements of spindle lengths in normal haploid yeast cells either 0 or 3 h after the shift to the restrictive temperature showed a broadly bimodal distribution; though the majority of spindles were between 0.5–1.5-μm long, representing cells in G1, S, and G2 phases of the cell cycle,

Figure 5. Double-labeled immunofluorescent staining of nup120A cells, showing that the NPC (green) were generally opposite the nucleoli (red) (A). The image is a computer generated composite from a single field of cells double labeled with a rabbit polyclonal anti-Nsp1p antibody (NPC protein; green) (EC10-2; Hurt, 1988) visualized with a fluorescein-conjugated donkey polyclonal anti-rabbit IgG antibody, and a monoclonal anti-Nop1p antibody (D77; Aris and Blobel, 1988) visualized with a Cy3-conjugated polyclonal donkey anti-mouse IgG antibody (nucleolar protein; red). The orientation of the NPC cap (NPC) in each cell was scored relative to the nucleolar position (No) (B) for a total of 200 cells. The relative orientations were divided into three classes as shown schematically: (a) opposite (~135°–225°); (b) adjacent (~45°–135° or ~225°–315°); and (c) coincident (~315°–45°) relative to the nucleolus (center at 0°). The histogram shows the number of cells in each class.
a minority (~20%) were much longer, between 3.0–6.0 μm, displaying cells between G2 and the end of M (Fig. 7; Byers and Goetsch, 1975; Winey et al., 1995). In contrast, nup120Δ cells appeared to be defective in their ability to properly form extended spindles even under semi-permissive conditions (which may reflect their longer doubling time) (Fig. 7). After 3 h at the restrictive temperature, only 3% of the spindles in nup120Δ cells exceeded 2.5 μm, compared with 23% in normal cells. Microtubule morphology in these cells was also abnormal; many cells contained unusually long cytoplasmic microtubules, and in some cells two clear, separate foci of microtubules could be seen, as if the spindle pole bodies had separated without forming a full spindle (Fig. 7). The frequency of budded cells in this population was also slightly higher than normal. These observations would suggest that the restriction causes a serious mitotic defect. A similar effect has been noted for a conditional mutation of the nuclear import receptor, SRPI (KAP60) (Loeb et al., 1995).

The effect of the temperature shift on the distribution of another nuclear marker, Nsr1p (Lee et al., 1991) was less obvious, although that proportion of the protein associated with the nucleoli reflected the progressive fragmentation observed with Nop1p, demonstrating that the phenomenon was not restricted to this marker, but was more likely a general effect on nucleolar architecture. Likewise, the distribution of chromatin within the nucleus did not seem grossly changed over the course of the temperature shift in the nup120Δ cells, as determined by DAPI staining (Figs. 6 A, 9, and 10).

Morphological Examination of nup120Δ Cells

The gross phenotypic effects of deletion of NUP120, as described above, are readily observed at the level of the light microscope. We therefore examined nup120Δ cells over a period of several hours following a shift to the restrictive temperature by thin-section electron microscopy in order to detect more subtle changes to the cellular ultrastructure. At the start of the shift, when the cells still exhibit their semi-permissive phenotype, the most striking morphological perturbations were the clusters of NPCs. These clustered structures were characterized by large islands of closely opposed NPCs spaced by short highly distorted regions of nuclear envelope (Fig. 8, 0h, arrows). However, in common with nup159ts mutant cells (Gorsch et al., 1995), nup145ΔN cells (Wente and Blobel, 1994), and nup133Δ cells (Li et al., 1995), NPCs could occasionally be found outside the clusters in apparently normal regions of the nuclear envelope (Fig. 8, 0h, arrowheads). One unique feature of nup120Δ cells was the degree of distortion of the spacer nuclear envelope in the NPC clustered regions, which was more extreme than that seen with either the nup159 or nup133 mutants but considerably less so than the nup145 mutant. Occasional small aggregates of electron dense material were also detected in the nucleoplasm (Fig. 8, a). After 3 h at the restrictive temperature, the clusters had evolved into a more elaborate lattice of cisternae and herniations interconnected by NPCs. These now strongly resembled the grape-like structures described for the nup145ΔN cells (Wente and Blobel, 1994). Like these, some very large herniations could be seen over one or more NPCs on the cytoplasmic side of the clusters (Fig. 8, 3h, arrows). Herniations were also seen in nup116Δ cells, forming a seal over the cytoplasmic face of the NPC (Wente and Blobel, 1992). The nup116Δ herniations were filled with an electron dense material which, by virtue of a peripheral punctate oligo(dT) fluorescence staining pattern seen in this same strain of cells, was identified as ribonucleoprotein. This was believed to have accumulated as a result of nuclear export through the NPC, filling the herniation with electron dense ribonucleoproteins. However, neither the nup120Δ nor the nup145ΔN herniations were filled with electron dense material, and at least in the case of the nup120Δ cells no peripheral punctate oligo(dT) staining was detected (see below). This may be due to the fact that NPCs could be found interconnecting the cluster’s cisternae and on their cytoplasmic faces, allowing the exchange of the cisternal contents with both the nucleoplasm and cytoplasm. Occasionally, adjacent nuclear membrane protuberances could be found curving towards each other (Fig. 8, 3h, arrowheads). It is possible that these were in the process of joining to form another cisterna, as proposed for the mechanism of herniation formation in the nup116Δ mutant. At 6 h the cells exhibited comparable or more extreme versions of the structures found at 3 h (some of the herniations were particularly large; Fig. 8, 6h, arrow), and at both time points clear examples of electron dense areas of the nucleoplasm could be seen. Similar aggregates of material in the nucleoplasm have been reported for the nup116Δ cells (Wente and Blobel, 1993) and for various RNA export mutants (Schneider et al., 1995).

Nuclear Transport at Restrictive Temperatures in nup120Δ Cells

Analysis of the distribution of poly(A)+ RNA in situ in nup120Δ was investigated using digoxigenin-labeled oligo(dT)30 followed by FITC-labeled anti-digoxigenin antibodies. There was no detectable accumulation of poly(A)+ RNA in the nucleus of nup120Δ cells at the semi-permissive temperature (23°C) or in wild-type cells at either 23° or 37°C. However within 1 h after the shift of nup120Δ cells to the restrictive temperature (37°C), poly(A)+ RNA...
began to accumulate within the nucleus of some cells, and by 3 and 6 h, most of the cells displayed the poly(A)$^+$ RNA export block (Fig. 9). The immunofluorescent signal was not localized to the periphery of the nucleus as observed for nup116$\Delta$ cells (Wente and Blobel, 1993) indicating that the mRNA does not accumulate in herniations of the nuclear envelope. The accumulation of poly(A)$^+$ RNA may be the primary effect leading to cell death in these cells as it occurs before the cells have stopped dividing and die (see Fig. 3).

Attempts to investigate the effect of nuclear import after the temperature shift failed to yield conclusive results. Using the nuclear protein Nsr1p as a marker (Lee et al., 1991) we could detect very little cytoplasmic accumulation of the immunofluorescent signal in nup120$\Delta$ cells as compared to wild-type cells after the shift to 37°C (data not shown). We therefore investigated the effects of temperature shift on the distribution of the shuttling import factor Kap60p (SRP1p) (Yano et al., 1992; Enenkel et al., 1995; Moroianu et al., 1995a). Although it has not been demonstrated in yeast, the mammalian homologue of Kap60p, karyopherin $\alpha$, has been shown to be imported into the nucleus coincident with the import of substrate and to shuttle out of the nucleus, presumably for the next round of import (Moroianu et al., 1995b; Görlich et al., 1995; Moroianu, J., and G. Blobel, unpublished observation). Indirect immunofluorescence microscopy using anti-Kap60p antibodies showed a distinct nuclear accumulation in nup120$\Delta$ cells after the temperature shift compared to wild-type cells where the protein, under the fixation conditions used here, appeared equally distributed between the nucleus and the cytoplasm (Fig. 10). The accumulation of Kap60p and poly(A)$^+$ RNA in the nucleus, combined with only a moderate effect on Nsr1p cytoplasmic accumulation is consistent with the idea that protein import remains relatively unaffected by the temperature shift, but that export of mRNA and Kap60p is significantly hindered.

Discussion

We have identified a novel nucleoporin we term Nup120p. This protein shows limited homology to Nup133p and no significant similarity to any other proteins in the data base. Nup120p (and its corresponding ORF, YKL057c) was identified by microsequence analysis of a protein ($M_\text{r} \sim 100,000$) present in a yeast subcellular fraction highly enriched in NPCs. Nup120p is present in this fraction in similar amounts to Nup133p (Rout and Blobel, 1993; Pemberton et al., 1995), but is not as abundant as Nic96p, Nup170p, Nup157p, Nup188p, or Pom152p (Aitchison et al., 1995). Immunofluorescent localization of a protein A-tagged Nup120p chimera and subcellular fractionation demonstrate that the protein is localized to the NPC. Furthermore, deletion of NUP120 causes a temperature sensitive block in poly(A)$^+$ RNA export, a wide spectrum of coincident morphological defects and constitutive NPC clustering. It is also synthetically lethal in combination with deletion of the genes encoding NUP170, NUP188, POM152, and NUP133.

The nuclear accumulation of poly(A)$^+$ RNA has been reported for mutations in the genes of several nucleoporins: NUP159 (Gorsch et al., 1995), NUP145 (Wente and Blobel, 1994), NUP133 (Li et al., 1995), NUP116 (Wente and Blobel, 1993), and NUP82 (Hurwitz and Blobel, 1995). This is likely to be the primary cause of the temperature-sensitive phenotype of nup120$\Delta$ cells, as there were no accompanying detectable nuclear import defects. Of course, the failure to detect cytoplasmic accumulation of endogenous nuclear proteins is not necessarily indicative of efficient nuclear import because mRNA export precedes protein synthesis. However, the accumulation of Kap60p (SRP1) in the nucleus after the temperature shift, coincident with the poly(A)$^+$ RNA export defect, suggests that the NPCs maintain import capabilities but have become export deficient. The RNA export defect may itself lead to several other pleiotropic effects on the morphology of the nucleus, although no obvious morphological phenotypes were coincident with a poly(A)$^+$ RNA export defect in nup82 cells (Hurwitz and Blobel, 1995).

Nucleolar fragmentation and dissociation from the nuclear envelope, although not reported for alterations in nucleoporins, has been observed in several RNA export mutants (reviewed in Schneiter et al., 1995) and may be a result of poly(A)$^+$ RNA accumulation (Kadowaki et al., 1994). Thus it is not surprising that an NPC protein whose loss causes a defect in poly(A)$^+$ RNA export may also cause nucleolar fragmentation. The structure of the nucleolus may be dynamically maintained by a balance in the nuclear import of material required for pre-ribosomal assembly and the export of pre-ribosomes and mRNAs required for component synthesis. Also, as discussed by Schneiter et al. (1995), the nucleolus itself may be involved in poly(A)$^+$ RNA export.

Abnormal spindle function as a potential consequence of defective nucleocytoplasmic transport has been reported for one other NPC-associated protein, namely the nuclear import factor Srp1p (Kap60p) (Loeb et al., 1995). This also resulted in an apparent mitotic defect, similar to that seen in nup120$\Delta$ cells at the restrictive temperature. Loeb et al. (1995) presented evidence that this defect was caused by an inability to import factors necessary for the degradation of key mitotic regulators, such as Clb2p. It is also possible that inefficient export of RNAs encoding other mitotically required proteins, such as MAPs and motor proteins, in turn causes the rapid cessation of cell division after the temperature shift.

The most dramatic morphological defect associated with the deletion of the NUP120 gene is NPC clustering. NPC clustering has also been reported for mutations in NUP145.
Figure 8. Electron micrographs of nup120A (nup120::URA3) cells at the permissive temperature (23°C) (0h) and after a shift to the restrictive temperature (37°C) for 3 and 6 h. The micrographs depict NPC clustering at both temperatures. At 23°C the clustered structures were characterized by large islands of closely opposed NPCs spaced by short highly distorted regions of nuclear envelope (0h, arrows); occasionally, isolated NPCs were also seen (0h, arrowheads). There is a great degree of distortion of the spacer nuclear envelope.
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Aitchison et al., 1995; Pemberton et al., 1995), and NUP159 (Gorsch et al., 1995). It is interesting to note that it is the region of Nup133p whose removal causes NPC clustering in nup133 cells that is most similar to a stretch in Nup120p. It has yet to be determined whether this homologous region on Nup120p is also responsible for maintaining the spatial distribution of NPCs in the nuclear envelope.

NPC clustering as a result of the loss of Nup120p occurs at both the semi-permissive and restrictive temperatures and is not linked to the observed transport defect. Demonstration that the clustering phenomenon does not itself cause cell death has also been shown for each of the other reported clustering mutants (Wente and Blobel, 1994; Doye et al., 1995; Pemberton et al., 1995). The morphological abnormalities associated with clustered NPCs observed here after a temperature shift of nup120Δ cells most closely resemble the grape-like clusters reported in nup145ΔN cells (Wente and Blobel, 1994). In both these strains, there are extensive nuclear envelope protrusions in the region of the NPC clusters. These clusters and envelope abnormalities in cells lacking Nup120p may reflect, as suggested by Wente and Blobel (1994), a disturbed connection of mutant NPCs with the pore membrane domain of the nuclear envelope, thus allowing fusion of the nuclear envelope over assembling NPCs. Not exclusive to this possibility is that NPC clustering may reflect a breakdown of NPC interaction with the nuclear or cytoskeletal matrix. As NPCs in wild-type cells are evenly distributed over the entire nuclear envelope (including that portion attached to the nucleolus), a uniform NPC distribution may be normally actively organized and maintained. This is supported by the extraordinary organization of NPCs found in cells from several higher eukaryotes including Xenopus, Equisetum, and Necturus (Maul, 1977; Akey, 1989). Loss of an NPC component disrupting this interaction (directly or indirectly) could therefore cause clusters of NPCs. The finding that NPC clusters in nup120Δ cells are generally found opposite the nucleolus may reflect the collapse of a higher order nuclear structure and diffusion of NPCs to a particular focal point in the nuclear envelope. Comparably, NPCs of spermatocyte nuclei are found clustered to one pole of the nuclear envelope in areas where the heterochromatin loops touch the nuclear envelope (Fawcett, 1981). It is unknown whether this structure is actively maintained or is a result of a lack of complex chromatin structure or nuclear matrix organization. Alternatively, the dynamics of the nuclear envelope itself could be affected by an altered structure of the pore membrane domain. This domain is believed to be the sole exchange site of membrane components between the inner and outer nuclear membranes and itself is tightly surrounded by NPC proteins (Akey and Rademacher, 1993; Wozniak et al., 1995). Perturbations in proteins surrounding or associated with this domain may in turn affect both its structure and the efficient exchange of materials between the two membranes. Thus, the clustering phenomenon may result from an alteration of the inner nuclear membrane composition (or lamina) or the local membrane environment surrounding each NPC.

It is curious that mutations in apparently dissimilar NPC components can lead to common morphological abnormalities, some of which are shared by nup120. So far, these would seem to fall into at least four broad classes, although obviously some overlap occurs. The first group includes genes which cause NPC clustering and ultimately the grape-like structures, as described here for nup120, are also observed in nup145 and nup133 cells. The second, seen in nup1 mutants and in various synthetic lethal combinations of nup170, nup188, and pom152, typically has cells with extreme convolutions and invaginations of the nuclear envelope, carrying seemingly normally spaced NPCs (Belanger et al., 1995; Aitchison et al., 1995; Nehrbass, U., S. Maguire, M. P. Rout, G. Blobel, and R. W. Wozniak, manuscript submitted). The third, displaying herniations which contain electron dense material over the cytoplasmic face of NPCs, is at the moment unique to nup116 cells. The fourth, like nup82, or individual deletions of NUP157, NUP170 (Aitchison et al., 1995), NUP188 (Nehrbass, U., S. Maguire, M. P. Rout, G. Blobel, and R. W. Wozniak, manuscript submitted), and POM152 (Wozniak et al., 1994), has no discernible structural phenotype.

The relatively distinct phenotypic classes described above would argue against the idea that their morphologies are the result of a pleiotropic effect caused by a general nuclear transport defect. It is not clear why the alteration or removal of such disparate proteins should cause these common phenotypes; perhaps those with similar phenotypes are components recruited at the same time in the sequential assembly of new NPCs, or are separate components of an NPC subcomplex with a defined set of functions, or affect the transport efficiencies of a subset of substrates. Alteration or removal of one component could lead to a failure in that step of NPC assembly, or produce a functionally compromised subcomplex. Either (or both) scenarios could in turn produce characteristic morphological perturbations, common to a particular pathway of assembly or transport. Furthermore, the severity of the morphological disturbances does not necessarily correlate with the cell viability. For example, the nup145ΔN mutant, with extreme convolutions of the nucleus, is relatively healthy; whereas the depletion of Nup82p causes no detectable morphological phenotype, but is lethal (Wente and Blobel, 1994; Hurwitz and Blobel, 1995).

The synthetic lethality caused by combination of nup120Δ with single deletions of NUP170, NUP188, or POM152 suggests a relatively specific genetic interaction. This does not imply direct physical interactions or overlap in the specific functions of these proteins, but may simply be a result
Figure 9. Poly(A)$^+$ RNA accumulates in the nucleus of \textit{nup120}$\Delta$ cells. In situ hybridization of oligo(dT)$_{30}$ in \textit{nup120}$\Delta$ cells (nup120::URA3) showed accumulation of poly(A)$^+$ RNA within the nucleus upon shift to the restrictive temperature, compared to wild-type cells (WT). The accumulation of poly(A)$^+$ RNA may be the primary effect leading to cell death in these cells as it occurs before the cells have stopped dividing and die (see Fig. 3). The digoxigenin-labeled oligo(dT)$_{30}$ was detected using fluorescein-conjugated antidigoxigenin antibodies. DAPI shows coincident staining of nuclear DNA. Bar, 5 μm.
The transport factor karyopherin α (Kap60p, SRP1) accumulates in the nucleus of nup120A cells (nup120::URA3), compared to wild-type cells (WT). Under these fixation conditions Kap60p is detected by immunofluorescence in the nucleus and cytoplasm of wild-type (WT) cells reflecting its shuttling between the nucleus and cytoplasm. Examination of (nup120::URA3) revealed similar distribution at permissive temperature (23°C) but Kap60p accumulation in the nucleus after a shift to the restrictive temperature (37°C) for 3 h. Kap60p was visualized using rabbit IgG antibody followed by Cy3-labeled donkey anti-rabbit IgG. DAPI shows coincident staining of nuclear DNA. Bar, 5 μm.

Figure 10. The transport factor karyopherin α (Kap60p, SRP1) accumulates in the nucleus of nup120A cells (nup120::URA3), compared to wild-type cells (WT). Under these fixation conditions Kap60p is detected by immunofluorescence in the nucleus and cytoplasm of wild-type (WT) cells reflecting its shuttling between the nucleus and cytoplasm. Examination of (nup120::URA3) revealed similar distribution at permissive temperature (23°C) but Kap60p accumulation in the nucleus after a shift to the restrictive temperature (37°C) for 3 h. Kap60p was visualized using rabbit IgG antibody followed by Cy3-labeled donkey anti-rabbit IgG. DAPI shows coincident staining of nuclear DNA. Bar, 5 μm.

of their presence in, and contributions to, the overall structure of the NPC. We assume that deletion of NUP120 compromises the structural integrity of the NPC resulting in the temperature sensitivity of nup120Δ cells and the associated phenotypes. Combining this defect with deletions of other nucleoporins, whether or not they themselves show an obvious phenotype, may further jeopardize the integrity of the NPC leading to its collapse. The situation becomes more difficult to interpret with the combination of nup120Δ with nup133Δ, since deletion of either on its own is deleterious to cell growth and viability. This synthetic lethal combination could equally be attributed to a combined effect on overall cell fitness, i.e., a result of the sick getting sicker. Because the NPC is a compact and complex organelle, with each NPC component likely interacting with many other NPC components (either indirectly or directly) to form the functional whole, numerous combinatorial synthetic lethal interactions would be expected. Therefore, such genetic interactions should not be overinterpreted in terms of the specific function of each nucleoporin.

The removal of Nup120p from the yeast NPC has extensive and varied effects on the nuclear morphology and function, which ultimately compromise cell growth and viability. As well as underscoring the intimate role NPCs play in
the active maintenance of eukaryotic cells, these results provide avenues to pursue concerning the interplay of apparently disparate processes in the cell life cycle.

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