Proteomic Analysis of Nucleoporin Interacting Proteins*

Received for publication, March 23, 2001, and in revised form, May 30, 2001
Published, JBC Papers in Press, May 31, 2001, DOI 10.1074/jbc.M102629200

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The Saccharomyces cerevisiae nuclear pore complex is a supramolecular assembly of 30 nucleoporins that cooperatively facilitate nucleocytoplasmic transport. Thirteen nucleoporins that contain FG peptide repeats (FG Nups) are proposed to function as stepping stones in karyopherin-mediated transport pathways. Here, protein interactions that occur at individual FG Nups were sampled using immobilized nucleoporins and yeast extracts. We find that many proteins bind to FG Nups in highly reproducible patterns. Among 135 proteins identified by mass spectrometry, most were karyopherins and nucleoporins. The PSFG nucleoporin Nup42p and the GLFG nucleoporins Nup49p, Nup57p, Nup100p, and Nup116p exhibited generic interactions with karyopherins; each bound 6–10 different karyopherin βs, including importins as well as exportins. Unexpectedly, the same Nups also captured the hexameric Nups48p complex and Nup2p. In contrast, the FXPG nucleoporins Nup1p, Nup2p, and Nup60p were more selective and captured mostly the Kap95p-Kap60p heterodimer. When the concentration of Gsp1p-GTP was elevated in the extracts to mimic the nucleoplasmic environment, the patterns of interacting proteins changed; exportins exhibiting enhanced binding to FG Nups, and importins exhibiting reduced binding. The results demonstrate a global role for Gsp1p-GTP on karyopherin-nucleoporin interactions and provide a rudimentary map of the routes that karyopherins take as they cross the nuclear pore complex.

The nuclear pore complex (NPC)1 forms an aqueous conduit between the cytoplasm and nucleoplasm of cells that allows passive diffusion of small molecules and facilitates transport of macromolecules (1). In Saccharomyces cerevisiae 30 distinct nucleoporins (Nups or nuclear pore complex proteins) interact to form an NPC, and each Nup is present in many copies per NPC (2). Mobile receptors termed karyopherin βs (importins, exportins, and transportins) recognize nuclear import or export signals of cargo molecules and facilitate nucleocytoplasmic transport by interacting with nucleoporins (3). Thirteen yeast nucleoporins that contain FG peptide repeats (FG Nups) (Fig. 1A) are proposed to function as stepping stones for karyopherin-mediated transport reactions (4), but the actual mechanism of protein transport across the NPC is unknown.

The GTPase Gsp1p binds directly to karyopherin βs and modulates their interaction with cargo and nucleoporins (4, 5). Because of the cytoplasmic location of Rna1p (the Gsp1p GTPase activating protein (GAP)) and the nucleoplasmic location of Prp20p (the Gsp1p guanine nucleotide exchange factor), it is generally assumed that the GTP-bound form of Gsp1p is more abundant in the nucleoplasm than in the cytoplasm, thus creating a gradient of Gsp1p-GTP across the nuclear envelope (6). A steep gradient of Gsp1p-GTP across the NPC is thought to drive nucleocytoplasmic transport. Indeed, Gsp1p-GTP binding to importins causes dissociation of their cargo whereas Gsp1p-GTP binding to exportins enhances their association with cargo (5). The effect of Gsp1p-GTP on karyopherin-nucleoporin interactions is less well characterized. In some cases, it has been shown that Gsp1p-GTP causes dissociation of importins from FG Nups (4, 7) and enhances association of exportins with FG Nups (8). Knowledge of all protein interactions that occur at each nucleoporin within the NPC would greatly enhance our understanding of the transport mechanism.

EXPERIMENTAL PROCEDURES

Preparation of Yeast Extracts—The yeast strain GPY60 was grown in 1 liter of YPD medium at 30 °C to a cell density of 2 × 10^6. Cells were harvested by sedimentation at 5000 × g for 10 min and resuspended in 20 mM Hepes, pH 6.8, 150 mM KOAc, 250 mM sorbitol, 2 mM Mg(OAc)₂, and protease inhibitors (0.1 mg/ml phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin, 2 μg/ml aprotinin, and 2 μg/ml leupeptin) to a final volume of 40 ml. Cells were passed through a French Press, and cell debris was removed by sedimentation at 30,000 × g for 30 min at 4 °C. The supernatant was filtered through a 0.45-μm filter (Millipore) and desalted in a Sephadex G-25 fine column (Amersham Pharmacia Biotech) pre-equilibrated in 20 mM Hepes, pH 6.8, 150 mM KOAc, 2 mM Mg(OAc)₂. Pooled fractions (~40 ml) were supplemented with 0.1% Tween, 2 mM dithiothreitol, and protease inhibitors as above. Aliquots were frozen in liquid nitrogen and stored at −70 °C. The concentration of protein in the extracts was typically 10 mg/ml as determined by Bradford assays using bovine serum albumin as a standard.

Preparation of Recombinant Proteins—Recombinant proteins (except His-Gsp1p) were expressed as glutathione S-transferase (GST) fusions using vector pGEX-2TK (Amersham Pharmacia Biotech). NUP2, NUP60, NUP57, NUP49, and NUP42 genes and portions of NUP100 (codons 1–640), NUP116 (codons 165–715), and NUP1 (codons 332–1076) encoding the entire or most of the FG repeat regions were amplified from yeast genomic DNA (Promega) using polymerase chain reaction. The polymerase chain reaction products were ligated into vector pGEX-2TK and transformed into the Escherichia coli strain BL21 Codon Plus (Novagen). The HIS-GSP1 (Q71L) expression plasmid was obtained from K. Weis (University of California, Berkeley, CA) and expressed in E. coli strain SG10039 (Qiagen). Cells were grown in 1 liter of 2× YT medium at 37 °C to a cell density of 1 × 10^8, with ampicillin (0.1 mg/ml) and 2% glucose for GST fusions or with 0.1 μg/ml ampicillin and 0.05 μg/ml kanamycin for His-tag fusions. Protein production was induced with 0.3 mM isopropyl-1-thio-β-D-galactopyranoside for 3 h at 24 °C. Cells were resuspended with 30 ml of chilled lysis buffer (50 mM...
Tris, pH 8.0, 100 mM NaCl, and 2 mM EDTA for GST fusions or 50 mM NaH$_2$PO$_4$, pH 8.0, 300 mM NaCl, 10 mM imidazole for His-tag fusions) and passed through a French Press (SLM Instruments). Cell debris was removed by centrifugation at 30,000 x g for 15 min at 4 °C, and TWEEN 20 (0.1%) was added to the supernatant. HIS-Gsp1p (Q71L) was purified on nickel-Sepharose beads according to the manufacturer’s instructions (Qiagen). The eluted fractions were purified further in a Superdex 75 gel filtration column (Amersham Pharmacia Biotech), pre-equilibrated with phosphate-buffered saline buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM NaH$_2$PO$_4$, 1.5 mM KH$_2$PO$_4$, pH 7.0) and supplemented with 0.1% TWEEN 20, 1 mM dithiothreitol, and 0.1 mM GTP. Aliquots of purified proteins were frozen in liquid nitrogen and stored at -70 °C.

**A**. Yeast FG Nucleoporins

**B**. Proteins in yeast extracts

**FIG. 1.** A, nucleoporins in *S. cerevisiae* that contain FG peptide repeats (FG Nups). Different types of FG peptides are depicted as colored ovals. SAFG, PSFG, and FG peptides are shown in pink, PSFG (alone) in green, SAFG (alone) in blue, GLFG in yellow, FXFG in red, and XXFG in gray. The sublocation of each nucleoporin in the structure of the NPC is also depicted. Note that nucleoporins containing the PSFG motif (PSFG Nups) reside on the cytoplasmic fibrils of the NPC, that GLFG Nups reside in the central transporter region, and that FXFG Nups (except Nup1p) reside in the nuclear basket structure of the NPC (2). B, fishing in yeast extracts. A cartoon depicting the assay used to capture FG Nup interacting proteins.

Many proteins bind to the immobilized FG Nups in highly reproducible patterns (Fig. 2, lanes 1). Bound proteins were captured from yeast extracts (and not from the *E. coli* extracts used to isolate the recombinant GST-Nups), as incubation of immobilized Nups with buffer alone did not contain proteins other than the bait (Fig. 2, lanes 2). Also, GST alone captured no proteins from *E. coli* or yeast extracts (data not shown). Sequential incubations of an extract with fresh Nup-coated beads (up to five times) yielded similar patterns of proteins bound (data not shown). Treatment of yeast extracts with RNase did not affect the interactions shown (data not shown).

Mass spectrometric analysis (MALDI-TOF and LC-MS) was used to identify all bound proteins greater than 60 kDa in size. This arbitrary cutoff was necessary for economy, and because we estimated that most proteins smaller than 60 kDa would be ribosomal proteins (the most abundant cargo in nucleocytoplasmic trafficking). Nups used as bait (most GST-Nups used were 60–80 kDa in size). The majority of proteins captured by the FG Nups were identified as karyopherins (49 of 135 total) and nucleoporins (32 of 135) (Figs. 2 and 3). Other proteins identified included cargos and potential cargos for karyopherins, mRNA-binding proteins, heat shock proteins, and a previously unidentified protein (Fig. 3). Although it is reasonable to assume that karyopherins bind directly to the immobilized FG Nups, it is less certain how all the other.
proteins interact with the immobilized Nups, directly or indirectly. A distinction between these two possibilities could be obtained in each case by testing the interaction with all purified proteins. For example, we confirmed that binding of Kap95p-Kap60p to each of the FG Nups is direct using stepwise reconstitution in solution binding assays with all purified proteins (data not shown). Proteins that resisted NaCl extraction were subsequently extracted with SDS (bottom panels). All protein fractions were resolved by SDS-polyacrylamide gel electrophoresis and stained with Coomassie Blue. The open circles in the gel frames represent molecular size markers at 200, 116, 97, and 66 kDa. All visible bands of proteins larger than 60 kDa were dissected from gels and processed for identification by mass spectrometry. Note the wide array of proteins bound to the different FG Nups and the significant effect that Gsp1p-GTP has on the binding patterns.

**Effect of Gsp1p-GTP on Nucleoporin Interacting Proteins**—In yeast extracts, Gsp1p is likely in its GDP-bound form as the absence of the nuclear envelope renders all Gsp1p-GTP vulnerable to action by Rna1p. Indeed, Gsp1p in the extracts co-purifies with Ntf2p as a 150-kDa complex in sizing columns and does not co-purify with karyopherins that fractionate in a range of sizes varying from 50 kDa (i.e. Kap123p) to 400

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**FIG. 2.** Yeast proteins captured on nucleoporin affinity resins and their identity as determined by mass spectrometry. GST-Nups (5 or 1 µg each) were immobilized on beads (10 µl packed) and incubated 3 h with buffer alone or with 1 ml of yeast extract containing ~10 µg of protein. In cases in which 1 µg of immobilized Nup was used (Nup60p, Nup2p, and Nup1p) the results were the same (yet more reproducible) as when 5 µg were used. Where indicated, extracts were supplemented with 30 µg of purified HIS-Gsp1p-GTP (Q71L). After washing of the beads, bound proteins were extracted with 1 M NaCl and collected by trichloroacetic acid-sodium deoxycholate precipitation (top and middle panels). Proteins that resisted NaCl extraction were subsequently extracted with SDS (bottom panels). All protein fractions were resolved by SDS-polyacrylamide gel electrophoresis and stained with Coomassie Blue. The open circles in the gel frames represent molecular size markers at 200, 116, 97, and 66 kDa. All visible bands of proteins larger than 60 kDa were dissected from gels and processed for identification by mass spectrometry. Note the wide array of proteins bound to the different FG Nups and the significant effect that Gsp1p-GTP has on the binding patterns.
kDa (i.e. Kap95p-Kap60p) (data not shown). As Ntf2p only binds the GDP-bound form of Gsp1p (12) and karyopherins only bind the GTP-bound form (4, 13), we conclude that Gsp1p in the extracts is in the GDP-bound form. Thus, the conditions used in the experiments described above likely mimic a cytoplasmic environment. To create a nucleoplasmic environment in the yeast extracts, the concentration of Gsp1p-GTP had to be increased. Addition of large amounts of Gsp1p-GTP, or Prp20p with GTP, had no effect on the patterns of proteins bound possibly because the Gsp1p GAP in the extracts (Rna1p) is abundant and rapidly converts all of the Gsp1p-GTP to Gsp1p-GDP. However, a mutant version of Gsp1p (Q71L) that cannot hydrolyze GTP and is resistant to action by Rna1p gave very different results.

Addition of mutant Gsp1p-GTP (Q71L) to yeast extracts (final concentration of 0.3% of total protein or 1.2 μM) had an effect on the patterns of proteins that bind FG Nups (Fig. 2, compare lanes 1 and 3) (for simplicity, Gsp1p Q71L is often referred to as Gsp1p throughout the text). In the majority of cases the change was very pronounced, but in a few cases the change was less dramatic yet always reproducible. Proteins exhibiting an increased ability to interact with the immobilized Nups were identified using mass spectrometry as before (upward pointing arrowheads) (Figs. 2 and 3). Proteins showing reduced binding in Gsp1p-GTP incubations (downward pointing arrowheads) were identified visually in cases when only one protein was present in the corresponding band in lane 1 and using Western blots with specific antibodies (data not shown) in cases where multiple proteins were present in the corresponding band in lane 1 (Figs. 2 and 3). Proteins with unaltered binding in the presence or absence of Gsp1p-GTP are unmarked, as are cases that we could not test. In general, we find
Protein Interactions at the Nuclear Pore Complex

FIG. 3. A comprehensive list of nucleoporin-interacting proteins detected here and the effects of Gsp1p-GTP on the interactions. Upward and downward pointing arrowheads mark interactions that were enhanced or reduced in the presence of Gsp1p-GTP, respectively. Asterisks mark proteins whose interaction (direct or indirect) with the corresponding FG Nup is also reported in the literature (20). Note that most of the interactions reported here are novel.

that exportins exhibited enhanced binding to FG Nups in the presence of Gsp1p-GTP, importins exhibited reduced binding in the presence of Gsp1p-GTP, and Nups exhibited no change in binding (except for Nup2p whose presence coincided with the importin Kap60p) (Fig. 2, A–H, compare lanes 1 and 3). All the results are summarized in Fig. 3 and discussed below in detail.

DISCUSSION

Karyopherins exhibited a wide range of binding interactions with the immobilized FG Nups, and in most cases Gsp1p-GTP modulated the interactions. These results support the hypothesis that Gsp1p-GTP promotes the association and dissociation of karyopherins and FG nucleoporins to facilitate transport of karyopherin-cargo complexes across the NPC (4). The fact that each FG Nup captured a highly reproducible set of proteins that included mostly karyopherins and FG nucleoporins and that interactions changed in a Gsp1p-GTP dependent manner, suggests that all binding interactions detected here are highly specific and may reflect the true occupancy of these nucleoporins at in vivo.

Karyopherin-nucleoporin interactions not detected here but detected elsewhere using more sensitive techniques (i.e. two-hybrid assays, overlay assays, Western blots, etc.) (20) may be too low in affinity to score well in our assay.

The exportins Msn5p and Crm1p were the most abundant karyopherins detected bound to immobilized Nups. Binding of Crm1p to FG Nups was significantly enhanced by Gsp1p-GTP (see Fig. 2B, for example), yet binding of Msn5p to the Nups was not, implying that Msn5p is not a stereotypical exportin. Indeed, Msn5p is unique among karyopherins in that it carries cargo in both directions across the NPC (14, 15). The observation that Kap120p associates best with FG Nups in the presence of Gsp1p-GTP (Fig. 2) provides biochemical evidence that it is an exportin, a suggestion previously made based on genetic data (16).

Surprisingly, no exportins were detected bound to the FG Nups of the nuclear basket structure (i.e. Nup1p, Nup2p, and Nup60p) (Figs. 2 and 3) even though these Nups are at the nucleoplasmic entryway of the NPC. In stark contrast, the exportin Crm1p binds tightly to Nup42p, a component of cytoplasmic fibrils at the opposite end of the NPC; their tight interaction resists extraction by 1 M NaCl (Fig. 2A, lane 3, bottom panel). In comparison, the binding avidity of Crm1p toward FG Nups of the central transporter region (i.e. Nup49p, Nup57p, Nup100p, and Nup116p) is clearly “intermediate” relative to the avidity of Crm1p for FG Nups of the nuclear basket or cytoplasmic fibrils (Fig. 2). Thus, it would appear that Crm1p binds FG Nups with increasing affinity as it moves along the NPC out of the nucleus (i.e. little or no binding to FG Nups in the nuclear basket, intermediate binding to FG Nups in the transporter region, and strong binding to FG Nups in the cytoplasmic fibrils). A similar hierarchy of binding sites provided by FG Nups is proposed to aid the movement of importins across the NPC but in the opposite direction (17, 18). In that model, Nups located in the nuclear basket structure would function as an “affinity trap” by providing the highest affinity docking sites for importins anywhere in the NPC (2). In our experiments, however, the three yeast nuclear basket Nups (Nup1p, Nup2p, and Nup60p) did not bind all importins with high affinity as predicted by that model. In fact, these nuclear basket Nups had an exclusive preference for Kap95p-Kap60p heterodimers and Kap123p in the case of Nup60p (Figs. 2 and 3). We therefore suggest that most importins (except Kap95p-Kap60p) do not use an affinity-trap mechanism at the nuclear basket structure to move directionally across the NPC.

It is currently unknown how importins of the karyopherin β family exit the nucleus, but it is generally assumed that each departs in a complex with Gsp1p-GTP. However, at least in the case of the importin Kap95p, Gsp1p-GTP interferes with its binding to most FG Nups when using all purified proteins in solution binding assays (data not shown). A similar situation occurs with mammalian components; binding of RanGTP to purified importin β blocks its binding to all FG Nups except Nup358 (19). It is therefore unclear how Kap95p-Gsp1p-GTP complexes interact with the NPC to move out of the nucleus. In that regard it is interesting to note that in the context of yeast extracts, binding of Kap95p to immobilized FG Nups was diminished (in most cases) but not prevented by the excess of Gsp1p-GTP added, even though the interaction between Kap95p and Kap60p was completely abolished (for the best example see Fig. 2E). Gsp1-GTP disrupts the Kap95p-Kap60p complex via direct binding to Kap95p (4). Thus, an auxiliary factor(s) in yeast extracts may stabilize binding of Kap95p-Gsp1p-GTP heterodimers to FG Nups such as Nup42p, Nup49p, Nup57p, Nup100p, and Nup116p (Fig. 2, A–E).

Four of the fourteen yeast karyopherin βIs (3) were absent from the list of proteins captured by the FG Nups. Missing were the importins Nmd5p and Kap114p and the exportins Cse1p and Los1p. Several possibilities can explain this unexpected result. The missing Kaps could have degraded in the yeast extracts, binding of Kap95p to immobilized FG Nups was diminished (in most cases) but not prevented by the excess of Gsp1p-GTP added, even though the interaction between Kap95p and Kap60p was completely abolished (for the best example see Fig. 2E). Gsp1-GTP disrupts the Kap95p-Kap60p complex via direct binding to Kap95p (4). Thus, an auxiliary factor(s) in yeast extracts may stabilize binding of Kap95p-Gsp1p-GTP heterodimers to FG Nups such as Nup42p, Nup49p, Nup57p, Nup100p, and Nup116p (Fig. 2, A–E).

The missing exportins Cse1p and Los1p could not be tested in the same manner as specific antibodies were unavailable. We favor the explanation that
Nmd5p, Kap114p, Cse1p, and Los1p were not captured simply because they exhibit low affinities toward the FG Nups ($K_d$ weaker than $1 \mu M$). In fact, we find that Cse1p binds Kap60p and Gsp1p-GTP with high affinity in solution binding assays with all purified proteins yet fails to bind stably to the immobilized FG Nups (with the exception of Nup116p to which it binds weakly) (data not shown). In that aspect, Nup116p was unique among the FG Nups tested as it also was the only nucleoporin that captured the importins Mtr10p and Pdr6p (Fig. 2E). Hence Nup116p may constitute the most promiscuous and generic docking site for karyopherins within the NPC.

The mRNA-binding proteins Mex67p and Pab1p were among the proteins captured by the FG Nups. Mex67p promotes mRNA export from the nucleus and binds FG Nups (21–23). Indeed, we detected Mex67p bound to Nup100p using mass spectrometry (Fig. 2D) and detected it bound to most other FG Nups using Western blots also with specific antibodies. A technical limitation made difficult the mass spectrometric identification of Mex67p in our experiments. This is because the interaction of Mex67p with FG Nups is resistant to extraction with $1 M$ NaCl but is disrupted efficiently with $1 M$ MgCl$_2$ or SDS. Unfortunately, these harsher extraction conditions also remove the GST-Nups from the beads which in turn mask any Mex67p present in the SDS eluates; Mex67p is similar in molecular size (67 kDa) to five of the eight GST-Nups used (60–80 kDa). Nevertheless, a small amount of Mex67p was extracted by NaCl from the FG Nups, and in the case of Nup100p, it was sufficient to detect it by mass spectrometry as the protein between Pab1p and Kap60p (Fig. 2D). A protein band at the same location is also visible in Nup49p, Nup57p, and Nup116p NaCl eluates and is likely Mex67p (Fig. 2B, C, and E; lanes 1). However, its presence in the NaCl eluates was scarce and variable making it difficult to identify by mass spectrometry. Pab1p is an abundant poly(A) RNA-binding protein required for the initiation of translation (24, 25). mRNAs did not mediate its binding to FG Nups (Fig. 2, lanes 1), as treatment of the yeast extracts with RNase did not diminish the amount of Pab1p bound (data not shown). As purified Pab1p does not bind directly to FG Nups (data not shown), its interaction is likely bridged by an unidentified factor.

The FG Nups can be categorized in three distinct groups based on their location within the NPC, their content of FG motifs, and the patterns of karyopherin binding. The PSFG Nups (containing SAFGXPSPFG motifs) are located in the cytoplasmic fibrils, the GLFG Nups are located in the central transporter region, and the FXFG Nups (except for Nsp1p) are located in the nuclear basket structure (Figs. 1 and 3) (2). In regard to karyopherin binding, the PSFG nucleoporin Nup42p and the central GLFG nucleoporins Nup49p, Nup57p, Nup100p, and Nup116p (Fig. 1) exhibited generic interactions with karyopherins; each band 6–10 different karyopherin βs including exportins as well as importins (Figs. 2 and 3). In contrast, the FXFG nucleoporins of the nuclear basket structure (Nup1p, Nup2p, and Nup60p) bound mostly Kap95p-Kap60p heterodimers (Fig. 2, F–H, lanes 1) and one additional importin (Kap123p) in the case of Nup60p (Fig. 2F). The apparent preference for Kap95p-Kap60p by FXFG Nups may be a consequence of the amino acid sequences surrounding the FG motif. In the FXFG Nups these regions are highly charged whereas in GLFG and PSFG Nups they are mostly uncharged. Alternatively, the binding specificity may be related to the presence of two phenylalanine residues in close proximity to each other: the FXF motif. Indeed, the phenylalanines in FXFG peptides are a dominant feature in the binding interface between importin β (vertebrate Kap95p) and a peptide containing FXFG peptide repeats (26).

The capture of the Nup84p complex by all GLFG and PSFG Nups (but not by FXFG Nups) was surprising (Fig. 2, A–E, and Fig. 3). The Nup84p subcomplex consists of the six proteins Nup84p, Nup85p, Nup120p, Sec13p, Seh1p, and the C terminus of Nup145p (cNup145p) that altogether play essential roles in nuclear pore biogenesis and nuclear export of polyadenylated RNA (27). Sec13p and Seh1p were not among the proteins identified here as they are smaller than 60 kDa, our arbitrary cutoff point for protein identification. Binding of the Nup84p complex to the immobilized Nups was not mediated by karyopherins as the amounts of Nup84p complex bound did not change (as karyopherins did) in experiments that contained Gsp1p-GTP (Fig. 2). It is attractive to speculate that the Nup84p complex interacts directly with FG repeat regions of Nups to move within the NPC much like karyopherins do. Alternatively, the Nup84p complex may perform a more structural role. It may be a component of the cytoplasmic and nucleoplasmic rings of the NPC that binds FG Nups to stabilize or manipulate their structure. Notably, the nucleoporin Nup133p and the Nup84p complex co-fractionate when binding FG Nups (Figs. 2 and 3) and when fractionated in sizing columns after extraction from Nup100p beads (data not shown); thus, we suggest that Nup133p is a component of the Nup84p complex.

It was also surprising to find Nup2p among the proteins captured by the FG Nups (Figs. 2 and 3). Its interaction with PSFG and GLFG Nups (and its modulation by Gsp1p-GTP) always coincided with the presence of Kap60p (Figs. 2 and 3). Binding of Nup2p to PSFG and GLFG Nups was mediated by Kap95p-Kap60p heterodimers as demonstrated by stepwise reconstitution in solution binding assays with all purified proteins (data not shown). In the case of Nup60p, however, its interaction with Nup2p is direct and is enhanced by Gsp1p-GTP (Fig. 2F).2 Altogether the results imply that Nup2p is a mobile nucleoporin that uses its interaction with Kap95p-Kap60p to move across the NPC.

Curiously Nup42p served as a stable docking site for Rna1p (the Gsp1p GAP) when the concentration of Gsp1p-GTP was increased (Fig. 2A, lane 3). The interaction between Rna1p and Nup42p was mediated by Crm1p as demonstrated by stepwise reconstitution in solution binding assays with purified proteins (data not shown). The fact that Rna1p has a nuclear export signal recognized by Crm1p (28) explains their interaction. The interaction between Rna1p, Crm1p, and Nup42p may provide a mechanism by which $S.~cerevisiae$ NPCs concentrate Rna1p at the cytoplasmic fibrils, much like vertebrate cells concentrate RanGAP (via SUMO-1) at Nup358, an FG Nup in cytoplasmic fibrils (29, 30). Rna1p was also detected bound to Nup49p, Nup57p, Nup100p, and Nup116p in a Gsp1p-GTP dependent manner but at reduced levels (data not shown); this is consistent with the proposal that Rna1p shuttles across the NPC (28).

The observation that Sec23p interacts with Nup42p and to a lesser extent with Nup116p (Fig. 2, A and E, lanes 3) is interesting because a genetic link between vesicular transport and the nuclear transport machinery has been reported (31). Sec23p is a structural component of COPII vesicle coats and functions as the Sar1p GAP (32); it also exhibits genetic interactions with Sec13p, which is a component of the Nup84p complex (33) and of COPII complexes (34). A binding partner of Sec23p, Sib2p/Lss1p (35), was also detected among the proteins bound to Nup42p (Fig. 2A, lane 3). We speculate that Sec23p modulates a putative interaction between the Nup84p complex and the nuclear pore membrane in a manner similar to its modulation of COPII binding to membranes (34).

2 D. Denning, B. Mykytka, N. P. C. Allen, L. Huang, A. Burlingame, and M. Rexach, submitted for publication.
The remainder of proteins identified included potential cargoes for karyopherins (the nuclear proteins Lpg1p, Nop8p, Rse1p, Smc2p, and Tfc1p), known cargoes for Kap104p (Nab2p and Nab4p) (36), several cytoplasmic proteins (Ded1p, Efr2p, Rpl2ap, Tef2p, and To1p), one protein of unknown function (Ydr101cp), one peripheral protein of the plasma membrane (Rpl2ap, Tef2p, and To1p), one protein of unknown function (Ydr101cp), and several heat shock proteins (Hsps). The presence of Ssa1p and Ssa2p Hsps among the proteins bound may be related to their auxiliary role in nuclear import reactions involving Kap95p-Kap60p heterodimers (38). Lastly, a few additional nucleoporins were detected bound to select FG Nups (i.e. Nup67p captured Nsp1p, Nup100p captured Nup188p and Nup170p, and Nup116p captured Nup60p). An interaction of Nup116p with Nup60p and Nup100p with Nup188p and Nup170p has not been previously reported; the interaction of Nsp1p with Nup67p is well documented (39).

Fig. 3 lists all protein interactions detected in our proteomic survey and denotes with an asterisk interactions previously reported in the literature. Note that most of the interactions discovered here are novel. This proteomic approach will be useful in the identification of additional networks of protein interactions and their dynamic response to cellular cues.

Acknowledgments—We thank Dr. Karsten Weis (University of California, Berkeley, CA) for providing the HIS-Gsp1p Q71L expression plasmid and Samir Patel for help preparing the figures for publication.

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