The Yeast Nucleoporin Nup2p Is Involved in Nuclear Export of Importin α/Srp1p*

James W. Booth‡‡, Kenneth D. Belanger‡, Maria I. Sannella‡, and Laura I. Davis‡**

From the ‡W. M. Keck Institute for Cellular Visualization, Rosenstiel Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02454 and the **Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710

The importin α/β heterodimer mediates nuclear import of proteins containing classical nuclear localization signals. After carrying its cargo into the nucleus, the importin dimer dissociates, and Srp1p (the yeast importin α subunit) is recycled to the cytoplasm in a complex with Cse1p and RanGTP. Nup2p is a yeast FXFG nucleoporin that contains a Ran-binding domain. We find that export of Srp1p from the nucleus is impaired in Δnup2 mutants. Also, Srp1p fusion proteins accumulate at the nuclear rim in wild-type cells but accumulate in the nuclear interior in Δnup2 cells. A deletion of NUP2 shows genetic interactions with mutants in SRP1 and PRP20, which encodes the Ran nucleotide exchange factor. Srp1p binds directly to an N-terminal domain of Nup2p. This region of Nup2p is sufficient to allow accumulation of an Srp1p fusion protein at the nuclear rim, but the C-terminal Ran-binding domain of Nup2p is required for efficient Srp1p export. Formation of the Srp1p-Cse1p-RanGTP export complex releases Srp1p from its binding site in Nup2p. We propose that Nup2p may act as a scaffold that facilitates formation of the Srp1p export complex.

Transport of macromolecules between the cytoplasm and nucleus in eukaryotic cells is mediated by soluble transport receptors. These receptors bind to specific protein or RNA cargoes and ferry them across the nuclear pore complex (NPC) (reviewed in Refs. 1 and 2). Several different nuclear import and export pathways have been identified (reviewed in Ref. 3). The first nuclear transport pathway that was described mediates import of proteins that contain a classical nuclear localization signal (NLS). The NLS is recognized by importin α/Srp1p, which forms a heterodimeric complex with importin β/Kap95p. By virtue of its interaction with importin β, importin α and its associated NLS-containing protein are carried through the NPC into the nucleus (5, 6). Binding of the GTP-bound form of the small GTPase Ran to importin β causes dissociation of the import complex in the nucleus (7, 8). Importin α/Srp1p then must be recycled to the cytoplasm to allow for multiple rounds of NLS protein import. Importin α/Srp1p is exported from the nucleus as part of a trimeric complex containing the exportin CAS/Cse1p and RanGTP (9–12). The export complex is ultimately dissociated in the cytoplasm by RanBP1/Yrb1p (13, 14) and RanGAP1/Rna1p (15, 16), acting to trigger GTP hydrolysis on Ran (9, 11).

All nuclear transport of macromolecules takes place through the NPC (17). The NPC consists of a large number of proteins termed nucleoporins (18). Many of the nucleoporins have repeated sequence motifs containing the dipeptide Phe-Gly (e.g. FXFG and GLFG). Numerous binding interactions have been identified between FG nucleoporins and soluble transport factors (7, 19–25). However, in most cases, the functional significance of these interactions is unclear. We previously showed that Srp1p binds to the yeast nucleoporins Nup1p and Nup2p (26). Nup2p is an FXFG nucleoporin that contains a Ran-binding domain (RanBD) homologous to that of RanBP1 (14, 27, 28). Nup2p is the only yeast nucleoporin that contains such a Ran-binding domain. In this report, we further investigate the interaction of Srp1p with Nup2p and its functional significance. Our results indicate that Nup2p is involved in the nuclear export of Srp1p and that Nup2p may act as a scaffold to facilitate formation of the Srp1p export complex at the NPC.

EXPERIMENTAL PROCEDURES

Reagents

Enzymes for molecular biology were purchased from New England Biolabs (Beverly, MA), Roche Molecular Biochemicals, and Amersham Pharmacia Biotech. 5-Fluoroorotic acid was obtained through the Genetics Society of America consortium. pRS vectors (29) were furnished by Dr. Phil Hieter. Rabbit-anti-β-galactosidase antibodies were obtained from Organon Technica (West Chester, PA), and anti-Xpress mAb was from Invitrogen (Carlsbad, CA).

Strains and Microbial Techniques

Unless otherwise specified, all yeast strains are isogenic with W303 (ade2-1 ura3-1 trp1-1 leu2-3, 112 his3-11, 15 can1-100 (Mata and Mata); a gift of Susan Wente). Yeast cell culture, medium preparation, and genetic manipulations were performed essentially as described (30). Yeast shuttle plasmids and linear fragments were introduced into yeast by lithium acetate transformation (31). DNA cloning was performed using standard techniques outlined by Sambrook et al. (32).

Disruption of NUP2

NUP2 was disrupted in W303 by transformation with a KAN° cassette (33) created by PCR using oligonucleotides 5′-CATACATCATTTTT-TCATACAGTCCCTGGTTAAACGAGTGTTGCTACGC and 5′-GGGTCAATCCTATTATTTTTAATTTGGTACTGGGACTAGTG-3′. This paper is available on line at http://www.jbc.org
GATCGT to generate LDY680 (Mata 2-Kan6). NUP2 was also disrupted with the TRP1 gene by transforming W303 with pJON133 (34), generating LDY626 (Mata 2-TRP1).

Genetic Crosses

NOY12 (srp1-31) (35) covered with pLDB283 (SRP1 URA3 CEN, pRS315 with an Hpa1-BgII fragment containing SRP1 cloned in at Smal- BamHI) was crossed to LDY627 (srp2-2 TRP1). From 28 tetrads dissected, 13 Trp+ spores were obtained that were 5-fluoroorotic acid sensitive at 37°C (Δsrp2 srp1-31). All of these were also 5-fluoroorotic acid sensitive at 25°C. LDY626 (Δsrp2 TRP1) was crossed to LDY551 (srp2∆20mtr1-1, second backcross of mtr1-1 from T127 (36) into W303), SSY11 (Δsrp1 100 (37)), and LDY544 (srp1∆20mtr1-1, second backcross of mtr1-1 from LDY431 (38) into W303). LDY 627 (srp2-2 TRP1) was crossed to SWY29 (Δsrp116 (37)). LDY 680 (Δsrp2-2 Kan6) was crossed to Y1705 (pC1 ΔI-1 (39)). LDY462 (nup2-2srp1-1) was crossed to srp2-2 TRP1.

Yeast Expression Plasmids

SRP1-GFP—SuperGlow GFP was cut out of pJK19–1 (40) with XhoI and inserted into the XhoI site of pRS305 (LEU2) to generate pLDB49. A promoterless SRP1 gene was amplified by PCR with oligonucleotides introducing a BamHI site at the start codon and an SpeI site before the stop codon and was inserted into pLDB349 at XhoI-SpeI to generate an in-frame fusion of SRP1 with GFP (pLDB520). pLDB520 was linearized with PstI, which cuts in SRP1, and transformed into W303 or LDY680 (Δsrp2-2 Kan6) to integrate at SRP1, generating JBY1 and JBY14, respectively.

SRP1-LacZ—A fusion of the SRP1 coding region to the Escherichia coli LacZ gene was created as follows. A fragment containing the SRP1 promoter and initiator methionine was amplified by PCR and cloned into YIp388R (41) to produce an in-frame fusion with LacZ. Next, the region of SRP1 encoding residues 463–542 was amplified and inserted into this plasmid. The resulting plasmid was linearized at a site between the SRP1 promoter and the C-terminal region, and transformed into W303. In vivo gap repair followed by integration of the vector resulted in the formation of full-length SRP1-LacZ fusions (transmembranes screened by Western blotting). The plasmid was rescued from this strain by cutting genomic DNA with PstI, followed by ligation and transformation into E. coli. The rescued plasmid (pLDB360) was cut with PstI and transformed into W303 or LDY680 (Δsrp2-2 Kan6) to integrate at SRP1, generating LDY970 and LDY969, respectively.

NUP2 Constructs—A Myc epitope was inserted after amino acid 428 of Nup2p by PCR mutagenesis of pJON76 (NUP2 URA3 CEN, pRS315 carrying a 6.1-kilobase BamHI fragment containing NUP2; I. N. Doetsch and S. I. Davis, unpublished data.) into pRS305 LEU2 to generate pLDB427. A promoterless NUP2 gene was constructed by amplifying BglII into W303. In vivo promoter and initiator methionine was amplified by PCR and cloned with SRP1 and Nup1p (778–1076) were described previously (26, 43). pLDB321, encoding Nup2p 1002–1041, was created by cloning an NsiI-Apal fragment of NUP2 into pJON133 (38). The rescued plasmid (pLDB360) was cut with EcoRI and PstI fragment from pLDB60 in pRS304 at EcoRI (pLDB683 (NUP2* TRP1, EcoRI fragment from pLDB60 at PstI) to generate pLDB427. The rescued plasmid (pLDB683) was cut with EcoRI and PstI and transformed into W303 or LDY680 (Δsrp2-2 Kan6) to integrate at SRP1, generating Nup2p Facilitates Srp1p Export.

Immunoufluorescence and Fluorescence Microscopy

Rabbit polyclonal anti-Srp1p antibodies were raised by injection with affinity-purified His6-Srp1p (28–542). The antibodies were affinity-purified from serum using His6-Srp1p (28–542) bound to nitrocellulose (46). Cells were treated for immunofluorescence essentially as described by Wente et al. (37) except that they were fixed for 12 min in buffer containing 10% formaldehyde and 10% methanol. Primary antibodies were used at a dilution of 1:200 and secondary antibodies at a dilution of 1:100. For visualization of Srp1p-GFP or Nup2p-HA, cells were grown overnight in selective medium and then diluted to OD600 = 0.1 and grown for 6 h in YPD to log phase. Cells were then harvested by centrifugation, washed once with phosphate-buffered saline, resuspended in ice cold 75% ethanol, incubated on ice for 10 min, and then washed once with phosphate-buffered saline and resuspended in phosphate-buffered saline containing DAPI (0.2 mg/ml) for viewing using a Nikon Optiphot microscope.

Solution Binding Assays and Protein Analysis

Binding experiments were performed in 0.5-ml siliconized tubes (Sigma) containing 100–200 μl of binding buffer (50 mM HEPES, pH 7.0, 200 mM NaCl, 50 mM KOAc, 10 mM MgOAc2, 0.1% Tween, 0.5 mM dithiothreitol, protease inhibitors (44) and 15 μl of glutathione-Sepharose beads with adsorbed GST fusion proteins. GST-Gsp1p adsorbed to glutathione-Sepharose beads was loaded with GTP by incubating for 30 min at 30°C in 5 mM EDTA, 20 mM potassium phosphate, pH 7.5, 2 mM dithiothreitol, 10 mM GTP, followed by addition of MgCl2 to a final concentration of 10 mM. Cleavage of GST-Gsp1p and GST-Nup2p (9–172) with thrombin (Sigma) was performed after precipitation with glutathione-Sepharose as described (47), followed by addition of a 1.5× molar excess of hirudin (Sigma). For analysis of Nup2p protein expression, yeast cell extracts were prepared by vortexing with glass beads in trichloroacetic acid (48). Western blotting was performed essentially as described (26) with either mAb 9E10 or mAb 414 as specific antibodies, both at a dilution of 1:1000.

Overlay of GST-Nups

Glutathione-Sepharose beads were used to precipitate GST-Nup fusion proteins from bacterial lysates. The beads were washed, and bound protein was eluted in SDS sample buffer containing 6 mM urea and subjected to SDS-PAGE without boiling. Two hundred nanograms of purified GST fusion was loaded in each lane, as determined by Coomasie Blue staining. Blot overlays were performed as described by Lee and Melese (49), using affinity-purified His6-Srp1p (74–542) as a ligand at a concentration of 10 μg/ml. Overlays were probed with anti-Xpress antibody diluted 1:5000, and bound antibody was detected using the ECL detection system (Amersham Pharmacia Biotech).

Precipitation of NLS-Human Serum Albumin (HSA)

HSA was coupled to oleogelipides consisting of the SV40 large T antigen NLS Pro-Lys-Lys22-Lys.Arg-Lys-Val or mutant peptides containing a substitution at position 5 (54). Purification of GST fusion proteins were bound to glutathione-Sepharose and washed one time with wash buffer (250 mM NaCl, 50 mM Heps, pH 7.0, 5 mM EDTA, 0.5 mM dithiothreitol, 0.1% Tween 20) before further additions. Incubations with Histidine-Srp1p (28–542) or NLS-HSA were performed in wash buffer plus 1% nonfat dry milk; washes were with wash buffer.

RESULTS

Srp1p Export Defect in Δsrp2 Mutants—The observation that Srp1p binds to Nup2p (26, 50) suggested that Nup2p

2 J. A. Hoffman and L. I. Davis, unpublished data.
yeast strains W303 (wild-type, a and b) or LDY680 (Δnup2, c and d) were grown at 30 °C and then prepared for immunofluorescence. Srp1p was localized by probing with polyclonal anti-Srp1p antibodies followed by DTAF-conjugated secondary antibodies (a and c). For comparison, nuclear DNA was visualized using DAPI (b and d). e–h, strains JBY1 (wild-type, e and f) or JBY14 (Δnup2, g and h) expressing Srp1p-GFP were fixed and viewed by fluorescence microscopy to detect the GFP signal (e and g) or DAPI (f and h). i–l, strains LDY970 (wild-type, i and j) or LDY969 (Δnup2, k and l) expressing Srp1p-LacZ were grown at 30 °C and then prepared for immunofluorescence and probed with anti-β-galactosidase antibodies followed by FITC-conjugated secondary antibodies (i and k), j and l, DAPI.

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As additional tools for studying Srp1p localization, fusions of Srp1p to either the green fluorescent protein (Srp1p-GFP) or β-galactosidase (Srp1p-LacZ) were constructed. These fusion proteins were expressed in yeast and localized by direct fluorescence (for Srp1p-GFP) or by immunofluorescence with anti-β-galactosidase antibodies (for Srp1p-LacZ). In wild-type cells, localization of either Srp1p-GFP (Fig. 1e) or Srp1p-LacZ (Fig. 1i) revealed a punctate fluorescence at the nuclear rim typical of NPC localization. This stands in contrast to the lack of nuclear rim accumulation of endogenous Srp1p (Fig. 1a). What-ever the reason for the accumulation of the fusion proteins, their localization in Δnup2 cells is markedly different, with both Srp1p-GFP (Fig. 1g) and Srp1p-LacZ (Fig. 1k) showing a uniform nuclear localization. Thus, as is seen with Srp1p, a lack of Nup2p causes nuclear accumulation of Srp1p fusion proteins. Furthermore, the lack of fusion protein accumulation at the nuclear rim in Δnup2 mutants suggests that a site critical for Srp1p binding at the NPC has been lost. Srp1p-GFP was able to complement an srp1::KAN6 disruption, showing that this fusion protein is functional; Srp1p-LacZ does not complement. Srp1p-GFP and Srp1p-LacZ were expressed at similar levels in wild-type and Δnup2 cells (data not shown).

No nuclear accumulation of Srp1p is seen in Δnup1 mutants (Fig. 2A), Δnup100 or Δnup116 mutants (data not shown), or numerous other nuclear transport mutants (11, 51). Moreover, deletion of NUP2 does not cause a general defect in nuclear export, as Crm1p-mediated nuclear export proceeds normally in the absence of Nup2p (Fig. 2B). Thus, the effects of loss of Nup2p on Srp1p export are specific.

Mapping of Srp1p-binding Regions in Nup2p and Nup1p—We previously showed that Srp1p interacts with Nup1p and Nup2p in yeast extracts (26). To determine whether the binding of Srp1p to Nup1p and Nup2p is direct, interactions between bacterially expressed fusion proteins were measured. To this end, regions of Nup1p and Nup2p were fused to GST, whereas amino acids 73–542 of Srp1p were expressed with an N-terminal His6 tag. Binding of His6-Srp1p (73–542) to
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Fig. 3. Discrete regions of Nup1p and Nup2p bind Srp1p. GST-Nup fusions were affinity-purified and then subjected to SDS-PAGE and transferred to nitrocellulose. Blots were overlaid with His6-Nup fusions were affinity-purified and then subjected to SDS-PAGE

![Graph](image)

lane

1. Nup2p
2. Nup2p(9-172)
3. Nup2p(182-546)
4. Nup1p(5-385)
5. Nup1p(142-385)
6. Nup1p(432-816)
7. Nup1p(778-1076)
8. Nup1p(778-999)
9. Nup1p(1002-1041)
10. Nup1p(1041-1076)
11. GST

The GST-Nup fusions were measured by gel overlay.

His6-Srp1p(73–542) binds directly to regions in both Nup1p and Nup2p (Fig. 3). A nup1 mutant has previously been shown to have a defect in Srp1p-mediated protein import (44). For Nup1p, the strongest Srp1p-binding region is found at the C terminus of the protein (Fig. 3, lanes 7, 9, and 10). Binding of Srp1p to the C terminus of Nup1p has also been reported by Floer et al. (52). We note that two nonoverlapping stretches at the C terminus both bind to Srp1p (Fig. 3, lanes 9 and 10), indicating the presence of multiple Srp1p binding sites. With Nup2p, Srp1p binds directly to the region N-terminal of the FXFG repeats (lane 2). A fusion of GST to the full-length Nup2p protein is highly degraded (lane 1), and Srp1p binds to the degradation products, which presumably represent a series of truncations of GST-Nup2p all containing the N terminus of the protein.

Srp1p does not bind to the FXFG repeat-containing regions of Nup1p or Nup2p (Fig. 3, lanes 3 and 6). Such repeats have been implicated in binding to importin β family members (7, 19, 21). We also note that the experiment shown in Fig. 3 was performed with Srp1p protein lacking the N-terminal 72 amino acids of Srp1p, which contain the IBB domain required for binding to Kap95p (5, 6). This demonstrates that binding of Srp1p to Nup1p and Nup2p does not require the IBB domain.

An identical pattern of binding affinities for the GST-Nup fusions was seen using a His6-tagged full-length Srp1p, although binding to all the Nups was weaker, possibly due to the presence of Srp1p breakdown products in the protein preparation (data not shown).

One possible reason for Srp1p binding to Nup1p and Nup2p is that it binds to NLS-like sequences in these nucleoporins. No NLS consensus sequences are found in the Srp1p-binding regions of Nup1p or Nup2p; however, to further address this possibility, we tested whether Srp1p could simultaneously bind to an NLS and to Nup1p or Nup2p by assaying for the ability of Srp1p to precipitate an NLS-HSA conjugate when bound to GST-Nup fusions (Fig. 4). His6-Srp1p (28–542) was incubated with GST-Nup fusions adsorbed to glutathione-Sepharose and then washed to remove unbound Srp1p. The Srp1p-Nup complex was then incubated with HSA conjugated to either a wild-type or mutant NLS peptide. Wild-type NLS-HSA was precipitated by each of the GST-Nup fusions when His6-Srp1p was present (Fig. 4, lanes 1 and 4) but was not detected in those samples to which His6-Srp1p was not added (lanes 2 and 5). A NLS-HSA conjugate containing a mutant NLS peptide was not precipitated (lanes 3 and 6). These data indicate that Srp1p can simultaneously bind to an NLS and to Nup2p or Nup1p. Because Srp1p has been shown to contain two adjacent NLS-binding sites (53), it is conceivable that these sites could mediate simultaneous binding to NLS-HSA and to an NLS in Nup1p or Nup2p; however, we consider this unlikely due to steric considerations. Thus, these results strongly suggest that binding of Srp1p to the nucleoporins is not via NLS-like sequences.

Domains of Nup2p Involved in Srp1p Export—In addition to the N-terminal Srp1p-binding domain and the central FXFG repeat domain, Nup2p contains a C-terminal RanBD (14, 28). To determine which domains are involved in Srp1p export, we constructed Nup2p truncations lacking the RanBD or the RanBD and the FXFG repeats and tested their ability to restore the wild-type pattern of Srp1p-GFP localization to a Δnup2 strain (Fig. 5A).

Expression of a full-length Myc-tagged version of Nup2p (Nup2pMyc) in Δnup2 cells restored the wild-type pattern of Srp1p-GFP fluorescence at the nuclear rim, with no accumulation in the nuclear interior (Fig. 5A, c). Expression of Nup2pMyc(1–546), lacking the C-terminal RanBD, resulted in increased fluorescence at the nuclear rim. However, significant nuclear accumulation of Srp1p-GFP remained relative to what was observed with full-length Nup2pMyc (Fig. 5A, c). Thus, Nup2p lacking the RanBD but containing the N-terminal Srp1p-binding domain appears to be sufficient to allow docking of Srp1p at the pore, but insufficient to restore wild-type levels...
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FIG. 5. Localization of Srp1p-GFP in cells expressing Nup2p truncations. A, Srp1p-GFP was visualized in the following Nup2p strains carrying the indicated integrated plasmids: JBY10 (empty vector, a), JBY12 (NUP2mcy, c), JBY16 (NUP2mcy(1–546), e), and JBY11 (NUP2(1–174), g). Arrows in g indicate cells showing fluorescence at the nuclear rim. Corresponding DAPI fields are shown in b, d, f, and h. B, cell extracts were made from wild-type cells (JBY1, lanes 1 and 4) or from Nup2p cells expressing Nup2p(1–546) (JBY12, lanes 2 and 5) or Nup2p(1–174) (JBY16, lane 3). Equal amounts of protein were subjected to SDS-PAGE, transferred to nitrocellulose, and probed with mAb 9E10 (left panel) to compare expression of Nup2p(1–546) and Nup2p(1–174) or with mAb 414 (right panel) to compare expression of Nup2p(1–546) with endogenous Nup2p. The arrow indicates the position of Nup2p. The top band in the right panel is Nup1p, and the bottom band is an unknown yeast protein recognized by mAb 414. The positions of the 116-, 97.5-, and 66-kDa molecular mass markers are indicated on the left.

of export. We confirmed that Nup2p(1–546) is expressed at the same level as full-length Nup2p(1–546) by Western blotting (Fig. 5B).

A further truncation of Nup2p, Nup2p(1–174), contains only the region N-terminal of the FXFG repeats. When this truncation was expressed in Δnup2 cells, Srp1p-GFP remained largely nuclear, but some fluorescence at the nuclear rim was still visible in many cells (Fig. 5A). The difference between Nup2p(1–174) and Nup2p(1–546) may indicate a role for the FXFG repeats in Srp1p export, or it may be that Nup2p(1–174) is expressed or targeted to the NPC to a lesser extent than Nup2p(1–546). In any case, these observations suggest that the N-terminal Srp1p-binding domain alone is sufficient to provide partial docking function, although export is very inefficient. Another indication that Nup2p(1–174) is partially functional comes from our observation that replacement of Srp1p with Srp1p-GFP results in slow growth in Δnup2 cells, but not wild-type cells, and that this growth defect is largely suppressed by expression of either Nup2p(1–174) or Nup2p(1–546). Thus, our observations indicate that the N-terminal Srp1p-binding domain of Nup2p is sufficient to provide partial function, but that efficient export requires the Ran-binding domain in addition.

Genetic Interactions—Independent evidence for a role for Nup2p in Srp1p export comes from genetic considerations. Deletion of NUP2 has no effect on growth (34), despite the observed defect in Srp1p export, implying that the residual level of Srp1p export is sufficient to sustain wild-type growth rates. If so, one might expect that deletion of NUP2 would have synergistic effects in combination with other mutants that affect the export or function of Srp1p.

To test this we crossed Δnup2 to several nuclear transport mutants. First, Δnup2 cells were crossed to cells bearing the srp1-31 temperature-sensitive mutation (35) and carrying wild-type SRP1 on a URA3-containing plasmid. Δnup2 srp1-31 haploid progeny were unable to grow on plates containing 5-fluoroorotic acid (Fig. 5A), indicating that they were unable to lose the SRP1 URA3 plasmid and thus that Δnup2 and srp1-31 are synthetically lethal. Moreover, as noted above, Δnup2 cells but not wild-type cells show a growth defect when endogenous Srp1p is replaced with Srp1p-GFP. These synthetic phenotypes would be expected if Nup2p plays a role in Srp1p export, because any defect in Srp1p function could be exacerbated by inhibition of its cycling between the nucleus and cytoplasm.

The Δnup2 mutation was also crossed to a temperature-sensitive mutant in PPR20/MTR1, the nucleotide exchange factor for Ran. mtr1-1 Δnup2 double mutants failed to grow at 30.5 °C, whereas mtr1-1 single mutants grew at this temperature (Fig. 5B). prp20-1 mutants, like Δnup2 mutants, have been shown to have a defect in Srp1p export (51), presumably because of a decrease in nuclear RanGTP, which is required for export complex formation. In contrast, we saw no obvious synthetic growth phenotype in Δnup2 cse1-1 double mutants, where cse1-1 is a cold-sensitive allele of the exportin Cse1p that carries Srp1p out of the nucleus (39). However, Δnup2 cse1-1 spores appeared to be slow to germinate (data not shown). The idea that Nup2p functions in export of Srp1p also suggests a possible explanation for the previously observed lethality of Δnup2 Δnup1 double mutants (34). As Δnup1 mutants are defective for Srp1p-mediated protein import (44), a decrease in the cytoplasmic pool of Srp1p in the absence of Nup2p may be lethal in the context of the Δnup1 mutation. Δnup2 showed no synergistic growth defects with rna1-1 or nup292 mutations (Fig. 5B) or with Δnup100 or Δnup116 (data not shown), suggesting that the interactions we observe with srp1p and prp20 are specific.

Comparison for Srp1p Binding—Srp1p export from the nucleus depends on its incorporation into a trimeric complex with Cse1p and RanGTP. To test this we crossed Δnup2 to several nuclear transport mutants. First, we confirmed that the recombinant Srp1p, Cse1p, and Gsp1p proteins were competent for forming trimeric export complexes in vitro (see Fig. 7A). GST-Gsp1p was bound to glutathione-Sepharose beads and loaded with GTP. Neither His6-Srp1p nor His6-Cse1p bound to GST-Gsp1p-GTP when added alone (lanes 1 and 2), but when added together, they bound efficiently (lane 3), consistent with the cooperative for-
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**DISCUSSION**

Our observations indicate that Nup2p is involved in Srp1p export, yet it binds to free Srp1p rather than to the Srp1p-Cse1p-RanGTP export complex. This suggests two possibilities for Nup2p function: either Nup2p acts at an early step in export, during export complex assembly, or at a late step, during complex disassembly. In the first case, Nup2p may facilitate formation of the Srp1p-Cse1p-RanGTP export complex at the NPC. Nup2p would provide a site to dock Srp1p, targeting complex formation directly to the NPC. In addition, docking of Srp1p and RanGTP near each other at two sites on Nup2p may improve the kinetics of assembly of the Srp1p-Cse1p-RanGTP complex. Export complex formation is a highly cooperative event in which all three components must
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assemble with each other, despite the fact that no two of the components interact pairwise in a stable manner. This process could be enhanced by bringing two of the binding partners into close proximity. Molecular scaffolds that bind to multiple proteins and thus facilitate their interaction are important in a number of systems (54, 55). Formation of the Srp1p-Cse1p-RanGTP export complex would release Srp1p from Nup2p (Fig. 7B), allowing subsequent translocation of the complex through the NPC. The coupling of complex formation to dissociation from Nup2p would prevent Nup2p from being an unproductive sink for these complexes. Instead, it could act catalytically to facilitate complex formation.

Alternatively, Nup2p might be involved in the terminal step of nuclear export, i.e. complex disassembly. The metazoan RanBD-containing nucleoporin RanBP2/Nup358 (56, 57), which is located on the cytoplasmic side of the NPC (58), and the cytosolic protein RanBP1/Yrb1p have been proposed to play such a role, acting to dissociate export complexes after their transit through the NPC by binding to RanGTP (59, 60).

Subsequent hydrolysis of GTP on the RanBD-bound Ran makes the dissociation irreversible (59). Nup2p may perform a similar function by competing RanGTP and Srp1p off Cse1p; however, we consider it more likely that Nup2p is involved in complex assembly than in disassembly. RanBP2 and RanBP1/Yrb1p bind RanGTP with high affinity, which is necessary for the proteins to function efficiently in complex disassembly. In contrast, Nup2p has a much lower affinity for RanGTP (28, 61). In this respect, it is similar to Yrb2p (62, 63). Yrb2p, like Nup2p, binds RanGTP with high affinity, which is necessary for the proteins to function efficiently in complex disassembly. The metazoan RanBD-containing nucleoporin RanBP2/Nup358 (56, 57), which is located on the cytoplasmic side of the NPC (58), and the cytosolic protein RanBP1/Yrb1p have been proposed to play such a role, acting to dissociate export complexes after their transit through the NPC by binding to RanGTP (59, 60).

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