A Nuclear Import Pathway for a Protein Involved in tRNA Maturation

Jonathan S. Rosenblum, Lucy F. Pemberton, and Günter Blobel

Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, New York 10021

Abstract. A limited number of transport factors, or karyopherins, ferry particular substrates between the cytoplasm and nucleoplasm. We identified the *Saccharomyces cerevisiae* gene YDR395w/*SXM1* as a potential karyopherin on the basis of limited sequence similarity to known karyopherins. From yeast cytosol, we isolated Sxm1p in complex with several potential import substrates. These substrates included Lhp1p, the yeast homologue of the human autoantigen La that has recently been shown to facilitate maturation of pre-tRNA, and three distinct ribosomal proteins, Rpl16p, Rpl25p, and Rpl34p. Further, we demonstrate that Lhp1p is specifically imported by Sxm1p. In the absence of Sxm1p, Lhp1p was mislocalized to the cytoplasm. Sxm1p and Lhp1p represent the karyopherin and a cognate substrate of a unique nuclear import pathway, one that operates upstream of a major pathway of pre-tRNA maturation, which itself is upstream of tRNA export in wild-type cells. In addition, through its association with ribosomal proteins, Sxm1p may have a role in coordinating ribosome biogenesis with tRNA processing.

The partitioning of nuclear from cytoplasmic matters is the defining feature of eukaryotes and separates the sites of transcription and translation. As a result, many proteins and RNAs need to be specifically transported into and out of the nucleoplasm. This transport takes place through the nuclear pore complex (NPC)[1] and is mediated by a structurally related family of soluble factors known as karyopherins (also named transportins, importins, or exportins). The karyopherin of the most widely studied import pathway is a heterodimer, composed of karyopherin α (Kap60p in yeast) and karyopherin β1 (Kap95p in yeast) (for reviews see Moroianu, 1997; Nigg, 1997). The Kap95p/Kap60p heterodimer, via Kap60p, binds to basic nuclear localization sequences (NLSs) in the cytoplasm and, via Kap95p, to peptide repeat-containing nucleoporins at the NPC. In a complex series of events, the trimeric complex is dissociated through the actions of p10 and the small GTPase Ran and its modulators (Rexach and Blobel, 1995, Nehrbass and Blobel, 1996). Kap60p and the NLS-containing protein continue into the nucleus, whereas Kap95p rapidly returns to the cytoplasm. Finally, the Kap60p/NLS interaction is disrupted (Rexach and Blobel, 1995, Moroianu et al., 1996), and Kap60p is recycled to the cytoplasm (Kutay et al., 1997; Moroianu et al., 1997). As much as 50% of all nuclear proteins have putative NLSs that may mediate nuclear import through the karyopherin α/karyopherin β1 pathway (Makkerh et al., 1996).

Additional noncompeting pathways to and from the nucleus for both proteins and RNAs have been directly or indirectly observed (Fischer et al., 1991; Garcia-Bustos et al., 1991; Michaud and Goldfarb, 1991, 1992). The completion of the yeast genome project has clarified and intensified the search for the molecular components of these pathways. Thorough characterization of these pathways should provide profound insight into how eukaryotic cells function. At the present time, it is not clear which classes of nuclear proteins will need their own transporters and how all of the transport pathways will be coordinated to permit efficient cellular function. Characterization of these pathways is in its early stages, as the molecular components for two additional import pathways and an export pathway have recently been identified in yeast (Aitchison et al., 1996; Rout et al., 1997; Stade et al., 1997). Each of these pathways is mediated by a protein similar in sequence to Kap95p. Importantly, none of the additional pathways appears to use Kap60p. Further characterization of these newly discovered transport factors will reveal additional similarities and differences to the Kap60p/Kap95p pathway. A limited number of additional open reading frames with modest similarity to Kap95p have been noted (Fornerod et al., 1997). One of these open reading frames is YDR395w, which was recently renamed *SXM1* (Seedorf and Silver, 1997). The following experiments were carried out to determine if Sxm1p is indeed a karyopherin, and if it is to determine its cargo.

Address all correspondence to Günter Blobel, Laboratory of Cell Biology, Howard Hughes Medical Institute, 1230 York Avenue, Box 168, The Rockefeller University, New York, NY 10021. Tel.: (212) 327-8096. Fax: (212) 327-7880. E-mail: blobel@rockvax.rockefeller.edu

1. *Abbreviations used in this paper*: DAPI, 4′,6-diamidino-2-phenylindole; GFP, green fluorescent protein; NLS, nuclear localization sequence; NPC, nuclear pore complex; PrA, protein A.

Materials and Methods

Yeast Strains and Methods

Strain DF5 was the parent strain for all those in this study (Finley et al., 1987), with the exception of the *LHP1* and *lhp1* strains in Fig. 5, which were generously provided by Christopher Yoo and Sandra Wolin (Yale University, New Haven, CT). Strains were manipulated and maintained according to standard methods (Rose et al., 1990).

Protein A Tagging and Gene Replacement

Protein A tagging and gene replacement of *SXM1* with *HIS3* were performed using published procedures (Aitchison et al., 1995). For protein A tagging, a PCR product was generated that contained a protein A, *HIS3*, *URA3* cassette flanked by the final 60 nucleotides of the coding region and 60 nucleotides downstream of the stop codon. *SXM1* was replaced with *HIS3* by generating a PCR product corresponding to the *HIS3* gene flanked by 60 nucleotides directly upstream and 60 nucleotides directly downstream of the coding region of *SXM1*. Yeast cells were transformed by electroporation. Correct integration was verified by PCR. Heterozygous diploids were sporulated and dissected to generate haploid strains.

Whole Yeast Protein Extracts

Protein extracts were prepared using a method similar to a published procedure (Yaffe and Schatz, 1984). Briefly, 1 ml of a saturated, overnight YPD culture was added to 140 μl of 1.85 M NaOH/7.4% 2-mercaptoethanol. After 2 min at room temperature, 140 μl of 50% (wt/vol) trichloroacetic acid was added. The mixture was spun for 5 min at 4°C. After aspiration of supernatant, the pellet was washed with 95% ethanol and dried. The pellet was then resuspended in 100 μl of sample buffer and heated at 95°C for 5 min. After centrifugation, 1–5 μl were subjected to SDS-PAGE.

Immunofluorescence

Indirect immunofluorescence was carried out as described (Rout et al., 1997). Briefly, yeast cells were fixed with 3.7% formaldehyde for 5 and 20 min. After digestion of the cell wall, spheroplasts were attached to glass slides. Protein A moieties were visualized by probing with rabbit IgG that had been preadsorbed to wild-type yeast spheroplasts (Cappel Laboratories, Malvern, PA) followed by Cy3-conjugated donkey anti–mouse IgG (Jackson ImmunoResearch, West Grove, PA). Nuclear DNA in fixed cells was visualized with 4′,6-diamidino-2-phenylindole (DAPI). Direct immunofluorescence was used to visualize live, unfixed cells transformed with a plasmid encoding an SV-40 NLS–green fluorescent protein (GFP) fusion (Shulga et al., 1996).

Cell Fractionation and Immunoisolation

Fractionation and immunoisolation of Sxm1–protein A (PrA) were as described (Aitchison et al., 1996). Briefly, postnuclear, postribosomal cytosol from 160 ml of a YPD culture with an OD_{600} of 1.6 was prepared. Rabbit IgG–Sepharose (Cappel Laboratories) was added to the cytosol. After overnight incubation, the Sepharose was extensively washed, and bound proteins were eluted with a step gradient from 50 to 4,500 mM $MgCl_2$. For mass spectrometry and microsequencing, eluted fractions were precipitated and resolved on a 10–20% acrylamide gel (Novex, San Diego, CA).

Northern Blot

Northern blot to analyze pre-tRNA processing was as described (Yoo and Wolin, 1997) with minor modifications. Briefly, total RNA from $\sim 3 \times 10^7$ cells was isolated using Tri Reagent (Sigma Chemical Co., St. Louis, MO) according to the manufacturer's instructions. This RNA was separated on a 10% TBE-Urea gel (Novex) and transferred in a tank apparatus to Hybond-N (Amersham Corp., Arlington, Heights). To assess overall RNA integrity, the samples were loaded in duplicate, and the half that was not transferred was stained with ethidium bromide and visualized with UV light. Oligonucleotides (Yoo and Wolin, 1997) and hybridization conditions (Tarn et al., 1995) have been described.

Overlay Assay

The overlay assay was carried out as previously described (Rout et al., 1997). Protein A fusion protein–containing cytosol was used analogously to antibodies, to probe blots for bands able to interact with the fusion protein. Briefly, cytosol was incubated overnight at 4°C with nitrocellulose blot. The blot was sequentially incubated with rabbit IgG (Cappel Laboratories) and HRP-conjugated goat anti–rabbit IgG (Amersham Corp.). Enhanced chemiluminescence was used for detection.

Results

Sxm1p Is Similar to Kap95p

Sxm1p is 16% identical and 50% similar to Kap95p (Fig. 1). When the two proteins are independently aligned, the similarity runs the length of the alignment. The common functions of the karyopherins are thought to be mediated by a more highly conserved amino-terminal domain; however, the boundaries of this domain are not obvious from our alignment. Although this similarity is modest, it is consistent with that seen for the similarity of other karyopherins, and proposed karyopherins, to Kap95p.

Genomic Tagging of SXM1 and Localization of Tagged Sxm1p

The carboxy terminus of the genomic copy of *SXM1* was directly fused, in frame, to the IgG-binding domains of *Staphylococcus aureus* PrA via integrative transformation into diploid cells. Sporulation and tetrad dissection yielded four viable colonies on YPD plates. The four strains produced grew at the same rate at 16, 30, and 37°C. Two of the four strains grew on plates lacking His and Ura. These strains also expressed a 140-kD protein reactive with rabbit IgG by immunoblotting, as expected for a fusion protein composed of Sxm1p and protein A.

The resulting fusion protein (Sxm1–PrA) was used to determine the subcellular distribution of Sxm1p by indi-

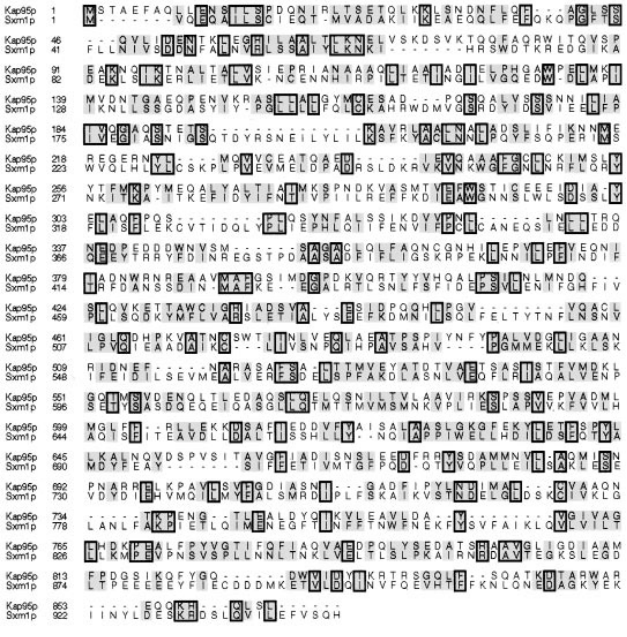

Figure 1. Kap95p and Sxm1p are homologous. The sequences were aligned by the Clustal method in MegAlign (DNA Star, Inc., Madison, WI). Similar amino acids are shaded, and identical amino acids are boxed and shaded.

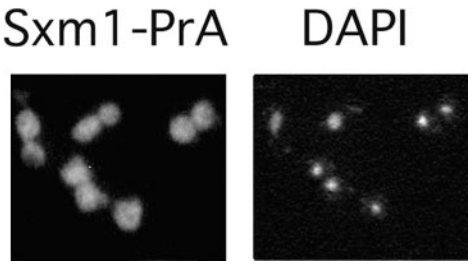

Figure 2. Sxm1–PrA is localized throughout the cell. The PrA moiety was visualized by indirect immunofluorescence. For reference, the right panel represents the nuclei of the same field of cells by staining for DNA with DAPI.

rect immunofluorescence microscopy. Using a fixation time of 5 min, Sxm1–PrA appeared predominantly in the nucleus (data not shown). This nuclear concentration is consistent with that seen for an Sxm1p–GFP fusion as reported by Seedorf and Silver (1997). After more prolonged fixation, however, the cytoplasmic pool of Sxm1–PrA appeared at least as concentrated as that in the nucleus (Fig. 2). Localization of Sxm1p in both the nucleus and cytoplasm is consistent with a role for it in nucleocytoplasmic transport.

Immunoisolation and Identification of Sxm1p-associated Proteins

If Sxm1p is indeed a factor involved in transporting newly synthesized proteins from the cytoplasm to the nucleus, some portion of the cytoplasmic pool of Sxm1p can be expected to be complexed with its nucleus-bound cargo. As such, we should be able to immunoisolate this transport complex from the cytoplasm of our Sxm1–PrA strain by virtue of the high affinity of PrA for IgG (Aitchison et al., 1996; Rout et al., 1997). We fractionated the Sxm1–PrA strain into crude nuclear and cytoplasmic fractions and subjected the cytoplasmic fraction to IgG-Sepharose chromatography. Four major Sxm1–PrA–associated proteins were eluted using either low pH (data not shown) or a magnesium chloride gradient (Fig. 3). The associated proteins eluted in the 100 and 250 mM $MgCl_2$ fractions, whereas the Sxm1–PrA/IgG interaction was not disrupted until ~1,000 mM $MgCl_2$. By Coomassie blue staining, there appeared to be similar concentrations of the associated proteins as compared with Sxm1–PrA, indicating that much of the cytosolic Sxm1–PrA exists complexed to these proteins.

After SDS-PAGE and staining with Coomassie blue, associated protein bands were individually excised from the gel and digested with endoprotease Lys-C. The resulting peptides were subjected to microsequencing (Fernandez et al., 1994) and/or mass spectrometry (Gharahdaghi et al., 1996). The 38-kD band was identified as Lhp1p, which is the yeast homologue of the human autoantigen La. This assignment was made by sequencing the peptide VIEALRSSEILEVSADGENV, which corresponds to amino acids 83–102 of Lhp1p. From the 22-kD band, the peptide EQLSGQTPVQ was sequenced, corresponding to amino acids 38–47 of the 60S ribosomal protein Rpl16p. Two other ribosomal proteins, Rpl25p and Rpl34p, were identified through mass spectrometry of the doublet centered at 15 kD.

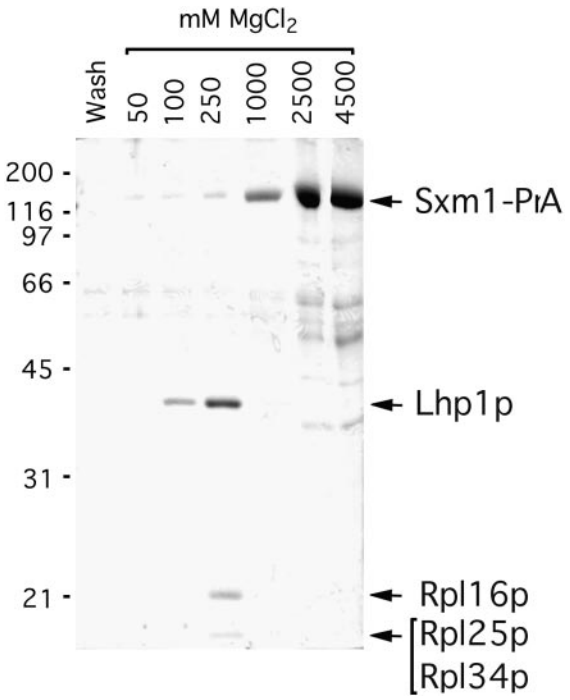

Figure 3. Immunoisolation of cytosolic Sxm1–PrA interacting proteins. Whole cytosol from an Sxm1–PrA strain was subjected to IgG-Sepharose chromatography. Fractions from the final wash and $MgCl_2$ gradient were separated by SDS-PAGE. Molecular mass standards are indicated to the left in kilodaltons. The gel was stained with Coomassie blue R.

Lhp1p, Unlike Other Nuclear Proteins, Is Mislocalized in a Strain Missing Sxm1p

An Sxm1p-deficient strain was generated by replacement of *SXM1* by the *HIS3* gene. After sporulation and tetrad dissection, all four spores were viable on YPD plates. No growth phenotype was observed for the *sxm1::HIS3* strain at any temperature. The absence of a growth phenotype is consistent with a previous study (Seedorf and Silver, 1997). Lhp1p, like the La protein of vertebrates (Simons et al., 1996), is concentrated in the nucleus at steady state. Like *SXM1*, *LHP1* is nonessential for growth (Yoo and Wolin, 1994; Lin-Marq and Clarkson, 1995). We characterized the impact of *SXM1* deletion on nuclear import of Lhp1p. As we had identified Lhp1p as a potential import substrate for Sxm1p, a dependence on Sxm1p for nuclear localization of Lhp1p would secure the definition of Sxm1p as a karyopherin. To examine the localization of Lhp1p, we constructed genomic Lhp1–PrA fusions in both a wild-type strain and in our *sxm1::HIS3* strain. As expected, Lhp1–PrA colocalized with DAPI-stained DNA in the nucleus of the wild-type strain (Fig. 4 *a*, *top*). In marked contrast, in the *sxm1::HIS3* strain, Lhp1-PrA was mislocalized to the cytoplasm, and in fact appears to be excluded from the nucleus in many cells (Fig. 4 *a*, *bottom*). Importantly, our *sxm1* strain retained its ability to import the endogenous nuclear protein Npl3p as well as a reporter bearing

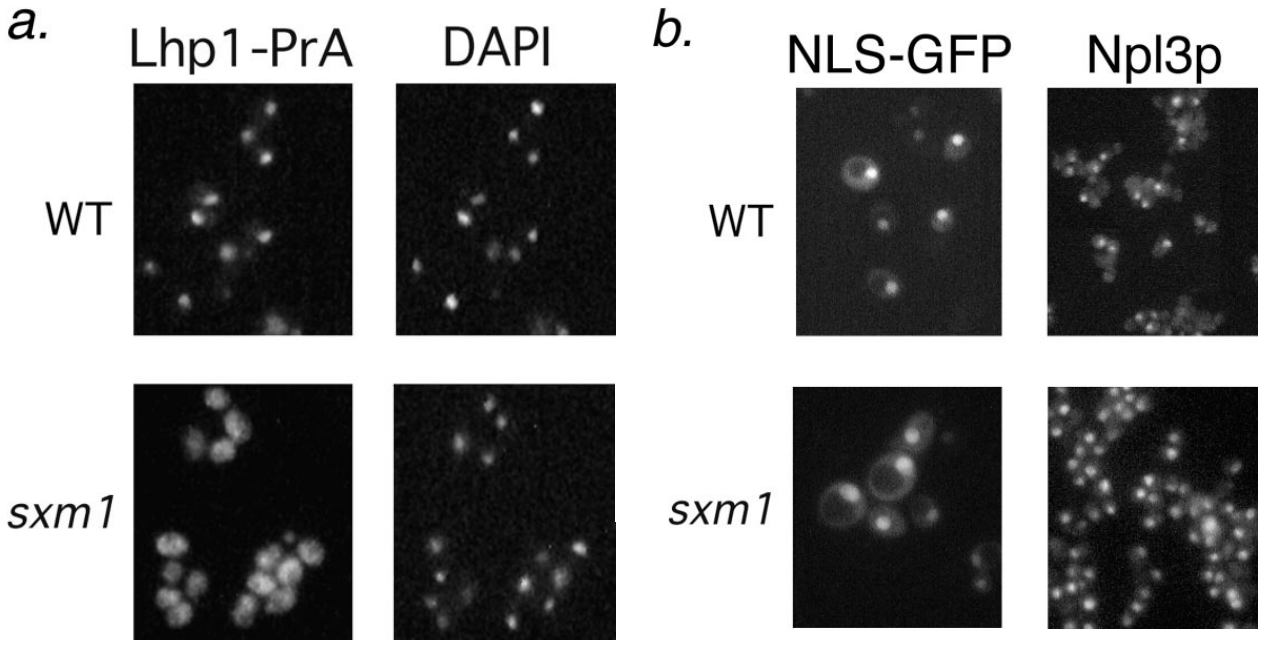

Figure 4. Deletion of *SXM1* leads to mislocalization of Lhp1-PrA but not other nuclear proteins. (*a*) Wild-type haploid cells (*top row*) and *sxm1* cells (*bottom row*), both with a genomic Lhp1-PrA fusion, are probed for Lhp1-PrA (*left*) and stained for DNA with DAPI (*right*). (*b*) Wild-type cells (*top row*) and *sxm1* cells (*bottom row*) were probed for either an NLS-GFP reporter (*left*) or the endogenous protein Npl3p (*right*).

the SV-40 large T nuclear localization sequence (Fig. 4 *b*). From these results, it is clear that Sxm1p is necessary for the nuclear import of Lhp1p and that this import operates in parallel to other nuclear import pathways.

Deletion of SXM1 Does Not Markedly Affect tRNA Processing

By virtue of their in vivo interactions with small RNAs, La and Lhp1p have been suggested to have a number of functions in RNA biogenesis (Gottlieb and Steitz, 1989; Bachmann et al., 1990; Meerovitch et al., 1993; Yoo and Wolin, 1997). The first direct effect of Lhp1p on small RNA biogenesis has recently been described. Lhp1p was shown to be essential *in vivo* for 3' endonucleolytic maturation of pre-tRNA (Yoo and Wolin, 1997). In the absence of Lhp1p, pre-tRNA was matured by an exonucleolytic pathway that does not appear to contribute to pre-tRNA maturation in wild-type cells (Yoo and Wolin, 1997). Maturation is important not only for the biogenesis of tRNA, but it is also a prerequisite for tRNA export, as unprocessed RNAs are retained in the nucleus (Haselbeck and Greer, 1993). To determine if our Lhp1–PrA fusion is functional and to characterize further phenotypes of the *SXM1* deletion, we analyzed maturation of $tRNA^{Ser}_{CGA}$ in our Lhp1–PrA strains that are wild type or deleted for *SXM1*. By Northern analysis of total RNA, it is possible to discern three distinct, intron-containing pre-$tRNA^{Ser}_{CGA}$ species in wild-type cells (Fig. 5, *A–C*; *D* is properly processed $tRNA^{Ser}_{CGA}$). The intermediates have 5′ and 3′ extensions (Fig. 5 *A*), only a 3′ extension (*B*), and no extension (*C*).

As previously described (Yoo and Wolin, 1997), deletion of *LHP1* leads to an alteration in precursors A and B; in our assay, both are dramatically reduced in abundance (Fig 5, compare lanes *1* and *2*). When the data are quantitated and normalized for intermediate C, whose abundance is independent of *LHP1* genotype (Yoo and Wolin, 1997) (Fig. 5, *bottom left*) the peaks representing intermediates A and B are both clearly smaller. Deletion of *SXM1* did not drastically affect the appearance of these intermediates for this pre-tRNA, although a minor effect was seen in precursor B (Fig. 5, compare lanes *3* and *4*; see also Fig. 5, *bottom right*). It is possible that even in the absence of Sxm1p, some Lhp1p is brought into the nucleus with low efficiency by another pathway. As Lhp1p is likely to act catalytically, inefficient transport or diffusion of Lhp1p into the nucleus could account for the appearance of intermediates A and B in the *SXM1*-deficient strain.

Like Other Karyopherins, Sxm1p Binds Components of the Nuclear Pore Complex

Having demonstrated in vivo that Sxm1p is necessary for both nuclear import and maximal activity of Lhp1p, we next tested the ability of Sxm1p to bind to known components of the yeast nuclear import machinery. As docking of transport complexes to the NPC is an essential step for import, we assayed the ability of Sxm1–PrA to bind either full-length or peptide-repeat regions of nucleoporins by a blot overlay assay. Bacterially expressed nucleoporins or nucleoporin fragments were separated by SDS-PAGE and transferred to nitrocellulose. Strips were overlaid with crude cytosol from our Sxm1–PrA strain. As a control, an adjacent strip was probed with cytosol from a Kap95–PrA strain. Like Kap95–PrA, Sxm1–PrA bound to full-length Nsp1p and the peptide-repeat domains of Nup159p and Nup1p. In contrast to Kap95–PrA, no binding of Sxm1–PrA to full-length Nup2p was seen (Fig. 6 *a*). As such, it

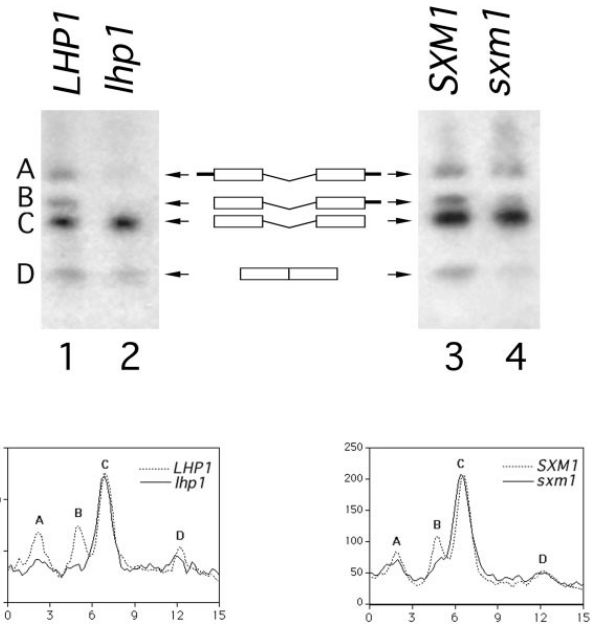

Figure 5. 3′ processing of pre-tRNA$^{Ser}_{CGA}$ Ser CGA in *LHP1*- and *SXM1*-deletion strains. A schematic of the intron-containing pre-tRNAs, species A, B, and C and of mature tRNA, species D, is included for reference. Exons are represented by boxes, 5′ and 3′ extensions are represented by black bars, and the central intron is shown. A Northern blot of total RNA was probed for intermediates A, B, and C using an oligonucleotide from the intron, and for mature tRNA using a probe that spans the properly spliced exons. Lane *1*, wild-type cells (*LHP1*); lane *2*, *lhp1* cells; lane *3*, Lhp1-PrA cells, wild-type for *SXM1* (*SXM1*); and lane *4*, Lhp1-PrA/*sxm1* cells (*sxm1*). The blot was quantified using a PhosphorImager with ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The graphs indicate radioactivity relative to distance, and peaks corresponding to intermediates are labeled. To account for minor loading discrepancies, the data in the graphs have been normalized to an internal control, precursor C, which was shown previously (Yoo and Wolin, 1997) to be independent of *LHP1* genotype.

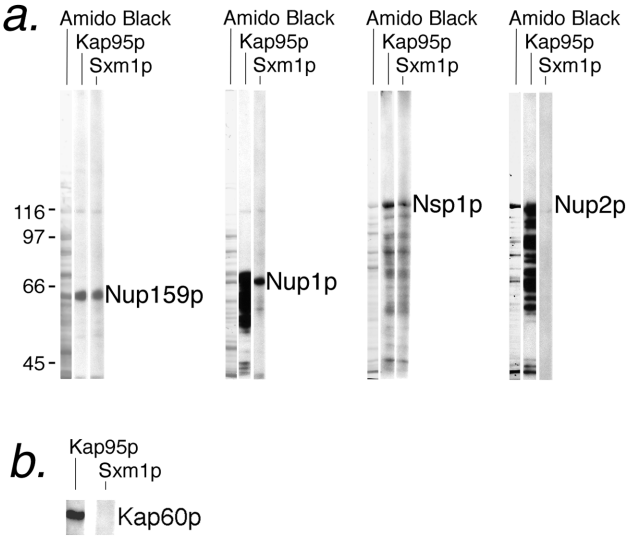

Figure 6. Sxm1–PrA binds to several nucleoporins but not to Kap60p. (*a*) Cytosol from either Kap95–PrA or Sxm1–PrA was overlaid on a nitrocellulose strip of electrophoretically separated crude *E. coli* lysate expressing repeat regions of Nup159p (amino acids 441–876; Kraemer et al., 1995) or Nup1p (amino acids 432–816; Rexach and Blobel, 1995) or full-length Nsp1p or Nup2p. A strip stained for total protein with amido black is included for reference. (*b*) Purified Kap60p was overlaid with Kap95-PrA cytosol or Sxm1–PrA cytosol.

appears that although both Sxm1p and Kap95p carry substrates through the NPC into the nucleus, they may each have distinct interactions along the way. In further experiments, Sxm1–PrA did not bind to either purified Kap60p (Fig. 6 *b*) or to purified Kap95p (data not shown) by overlay blot. As Sxm1–PrA does not appear to bind nucleoporins via Kap95p or substrates via Kap60p, Sxm1p most likely falls into the class of β karyopherins that includes Kap104p and Kap123p. The members of this class of karyopherins bind directly to both nucleoporins and to transport substrates.

Discussion

In this study, we have shown that Sxm1p is a karyopherin and identified one of its major transport substrates. Lhp1p was shown to bind to Sxm1p in cytosol, a compartment where the complex between an import substrate and its karyopherin is expected to be stable by virtue of a low concentration of Ran-GTP. Furthermore, we observed that in a strain deleted for *SXM1*, Lhp1p was mislocalized to the cytoplasm, apparently unable to enter the nucleus. Significantly, the localization of other nuclear proteins was unaffected, suggesting that the Sxm1p pathway operates in parallel to previously characterized pathways. In addition to Lhp1p, three ribosomal proteins were isolated in complex with Sxm1p. It is possible that Sxm1p is able to mediate the import of these ribosomal proteins. As for Lhp1p, the import of the ribosomal proteins may be the result of either direct or indirect interaction with Sxm1p. Finally, we showed that Sxm1p is able to bind in vitro to peptide-repeat–containing nucleoporins, but not to Kap60p. Sxm1p, therefore, is a β karyopherin able to bind transport substrates and to dock at the NPC.

A Nuclear Import Pathway for Lhp1p

The La autoantigen directly binds all nascent RNA polymerase III transcripts (Stefano, 1984). In vivo, La is found in ribonucleoprotein particles with many classes of small RNAs, including pre-tRNA and U6 RNA, as well as the 5S rRNP (Hendrick et al., 1981; Rinke and Steitz, 1982, 1985). Specific nuclear retention and export of several classes of RNAs have been suggested to be mediated by La (Guddat et al., 1990; Boelens et al., 1995; Grimm et al., 1997). Lhp1p, the yeast homologue of La (Lin-Marq and Clarkson, 1995, Yoo and Wolin, 1994), was recently shown to be essential for accurate pre-tRNA processing (Yoo and Wolin, 1997). In wild-type cells, the 3′ end of tRNA is determined by an endonucleolytic cleavage facilitated by Lhp1p (Yoo and Wolin, 1997). As *LHP1* is nonessential, it is evident that an alternative, exonucleolytic pathway can also produce mature, exportable tRNA. The relative kinetics and downstream events from each of these path-

ways is unknown. As Lhp1p binds to all RNA polymerase III transcripts and not just pre-tRNAs, it is likely to have additional roles in RNA biogenesis (Yoo and Wolin, 1997). Many of these other functions of Lhp1p are likely to be carried out in the nucleus as well. As a result, factors influencing the import of Lhp1p may be used to modulate its activities.

The human La protein (hLa) has recently been shown to contain a nuclear localization sequence capable of leading to the import of a normally cytosolic reporter (Simons et al., 1996). The hLa NLS bears several similarities to the consensus bipartite NLS (Dingwall and Laskey, 1991; Makkerh et al., 1996). One major difference, however, is the presence of several acidic residues in the hLa NLS. This NLS may have been assumed to mediate import via the karyopherin α/β1pathway. However, extrapolating from our data in yeast, it may be assumed that mammalian cells also have a parallel import pathway for this highly conserved protein mediated by a Sxm1p-type karyopherin. In this light, it should be noted that all of the proteins that we isolated in complex with Sxm1p have homologues in higher eukaryotes.

A Backup Pathway for the Import of Ribosomal Proteins

Like *KAP123*, both *SXM1* and *LHP1* are nonessential in yeast. Even so, the benefits of the Sxm1p pathway have outweighed its metabolic cost. *SXM1* was originally isolated in a screen for genes able to complement the conditional loss of Pse1p (Seedorf and Silver, 1997). Kap123p has recently been shown to be the primary carrier of ribosomal proteins into the nucleus, possibly backed up by Pse1p (Rout et al., 1997). Interestingly, Seedorf and Silver (1997) have generated a strain lacking Kap123p with a conditional allele of Pse1p that rapidly accumulates nuclear mRNA at 36°C. Overexpression of *SXM1* in this strain complements its growth defect and partially restores normal mRNA export (Seedorf and Silver, 1997). On the basis of having a phenotype relating to a defect in the export of macromolecules from the nucleus, Sxm1p has been proposed to be an export factor (Ullman et al., 1997).

Our results do not address the possibility that Sxm1p may have a role in the export of macromolecules from the nucleus. However, from our data we can conclude that Sxm1p is necessary for the import of Lhp1p. In addition, it is possible that the Sxm1p pathway also is able to import ribosomal proteins Rpl16p, Rpl25p, and Rpl34p. It has recently been shown that various karyopherins overlap in their ability to import ribosomal proteins (Rout et al., 1997). In fact, one of the ribosomal proteins that we isolated in complex with Sxm1p, Rpl16p, has also been isolated in complex with Kap123p (Rout et al., 1997). It is therefore likely that in cells lacking a functional Sxm1p import pathway, Rpl16p, Rpl25p, and Rpl34p would be imported by Kap123p or Pse1p. Likewise, it is possible that the genetic interaction seen by Seedorf and Silver (1997) between *KAP123*/*PSE1* and *SXM1* results not from an overlap in their ability to export mRNA but from their ability to import ribosomal proteins. Indeed, the accumulation of mRNA in the nucleus is a common phenotype, shown in one study to affect 2% of all temperature-sensitive strains examined (Kadowaki et al., 1994). 16 complementation groups were found, and the screen does not appear to be saturated (Kadowaki et al., 1994). Various modulators of nucleocytoplasmic transport have been shown to be associated with mRNA export phenotypes, including components of the NPC and regulators of the small GTPase Ran (Kadowaki et al., 1994; Doye and Hurt, 1997). In many of these cases, the RNA export phenotype is not a primary phenotype.

The Sxm1p Pathway May Coordinate tRNA Processing/Export with Ribosome Biogenesis

We have described a nuclear import pathway, one of whose substrates is a protein required for a step in pre-tRNA maturation in wild-type cells. This 3′ maturation occurs via an endonucleolytic cleavage event. As *LHP1* is not an essential gene, it is clear that the exonucleolytic processing of pre-tRNAs that occurs in its absence also produces mature, exportable tRNA. As tRNA maturation is upstream from export, it is possible that the karyopherin that we have characterized is involved in this process in Lhp1p-containing cells as well. Lhp1p binds in vivo to several other classes of small RNAs and thus may also be involved in their processing or localization. The convergence of the pathway to import Lhp1p and that which may import several ribosomal proteins could not have been predicted. However, this convergence is likely to be physiologically relevant. In this light, it should be noted that studies in *Xenopus* have implicated a relay of La and a ribosomal protein in the export of 5S rRNA (Guddat et al., 1990). Newly transcribed, nuclear 5S rRNA was seen to transiently bind the *Xenopus* La, followed by binding to the ribosomal protein L5. The L5/5S RNP then exited the NPC and could be observed in the cytoplasm. It is possible that the ribosomal proteins that we have isolated in complex with Sxm1p perform a similar function in accompanying Lhp1p-interacting RNAs out of the nucleus.

A further physiological role for the Sxm1p transport route may be to coordinate the processing and export of tRNA with ribosome biogenesis. As tRNA processing is an essential step that precedes export, the kinetics of processing may be capable of regulating export rate. Export of mature tRNA is extremely fast; in amphibians, about 2×10^9 molecules of a particular tRNA are exported per minute per nucleus (Zasloff, 1983). It is possible that regulation of the synthesis or activity of Sxm1p could affect the quantity of Lhp1p transported to the nucleus. Kinetic control of tRNA maturation could be achieved by controlling the contribution of two pathways, endonucleolytic and exonucleolytic, to 3′ tRNA processing, a process in which Lhp1p has been implicated (Yoo and Wolin, 1997). Furthermore, the potential Sxm1p substrate Rpl16p has a role in ribosome biogenesis (Rotenberg et al., 1988; Moritz et al., 1990, 1991). Therefore, Sxm1p may serve as a unifying factor in the coordination of tRNA export and ribosome assembly.

We thank Joe Fernandez and Farzin Gharahdaghi of the Rockefeller University Protein/DNA Technology Center for protein sequencing and mass spectral analysis. We are grateful to Christopher Yoo and Sandra Wolin for providing the *lhp1* and *LHP1* strains, John Aitchison and Mike Rout for Kap95-PrA cytosol, and Ulf Nehrbass, Monique Floer, and Michael Rexach for bacterially expressed proteins. The advice and suggestions of members of the Blobel lab are gratefully appreciated.

J.S. Rosenblum was supported by a postdoctoral fellowship from the National Institutes of Health.

Received for publication 10 October 1997 and in revised form 7 November 1997.

References

Aitchison, J.D., M.P. Rout, M. Marelli, G. Blobel, and R.W. Wozniak. 1995. Two novel related yeast nucleoporins Nup170p and Nup157p: complementation with the vertebrate homologue Nup155p and functional interactions with the yeast nuclear pore-membrane protein Pom152p. *J. Cell Biol.* 131: 1133–1148.

Aitchison, J.D., G. Blobel, and M.P. Rout. 1996. Kap104p: a karyopherin involved in the nuclear transport of messenger RNA binding proteins. *Science*. 274:624–627.

Bachmann, M., K. Pfeifer, H.C. Schroder, and W.E. Muller. 1990. Characterization of the autoantigen La as a nucleic acid-dependent ATPase/dATPase with melting properties. *Cell*. 60:85–93.

Boelens, W.C., I. Palacios, and I.W. Mattaj. 1995. Nuclear retention of RNA as a mechanism for localization. *RNA*. 1:273–283.

Dingwall, C., and R.A. Laskey. 1991. Nuclear targeting sequences—a consensus? *Trends Biol. Sci.* 16:478–481.

Doye, V., and E. Hurt. 1997. From nucleoporins to nuclear pore complexes. *Curr. Opin. Cell Biol.* 9:401–411.

Fernandez, J., L. Andrews, and S.M. Mische. 1994. An improved procedure for enzymatic digestion of polyvinylidene difluoride-bound proteins for internal sequence analysis. *Anal. Biochem.* 218:112–117.

Finley, D., E. Ozkaynak, and A. Varshavsky. 1987. The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell*. 48:1035–1046.

Fischer, U., E. Darzynkiewicz, S.M. Tahara, N.A. Dathan, R. Luhrmann, and I.W. Mattaj. 1991. Diversity in the signals required for nuclear accumulation of U snRNPs and variety in the pathways of nuclear transport. *J. Cell Biol.* 113:705–714.

Fornerod, M., J. van Deursen, S. van Baal, A. Reynolds, D. Davis, K. G. Murti, J. Fransen, and G. Grosveld. 1997. The human homologue of yeast CRM1 is in a dynamic subcomplex with CAN/Nup214 and a novel nuclear pore component Nup88. *EMBO (Eur. Mol. Biol. Organ.) J*. 16:807–816.

Garcia-Bustos, J.F., P. Wagner, and M.N. Hall. 1991. Nuclear import substrates compete for a limited number of binding sites. Evidence for different classes of yeast nuclear import receptors. *J. Biol. Chem.* 266:22303–22306.

Gharahdaghi, F., M. Kirchner, J. Fernandez, and S.M. Mische. 1996. Peptide-mass profiles of polyvinylidene difluoride-bound proteins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the presence of nonionic detergents. *Anal. Biochem.* 233:94–99.

Gottlieb, E., and J.A. Steitz. 1989. The RNA binding protein La influences both the accuracy and the efficiency of RNA polymerase III transcription in vitro. *EMBO (Eur. Mol. Biol. Organ.) J*. 8:841–850.

Grimm, C., E. Lund, and J.E. Dahlberg. 1997. In vivo selection of RNAs that localize in the nucleus. *EMBO (Eur. Mol. Biol. Organ.) J*. 16:793–806.

Guddat, U., A.H. Bakken, and T. Pieler. 1990. Protein-mediated nuclear export of RNA: 5S rRNA containing small RNPs in Xenopus oocytes. *Cell*. 60: 619–628.

Haselbeck, R.C., and C.L. Greer. 1993. Minimum intron requirements for tRNA splicing and nuclear transport in Xenopus oocytes. *Biochemistry*. 32: 8575–8581.

Hendrick, J.P., S.L. Wolin, J. Rinke, M.R. Lerner, and J.A. Steitz. 1981. Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. *Mol. Cell. Biol.* 1:1138–1149.

Kadowaki, T., S. Chen, M. Hitomi, E. Jacobs, C. Kumagai, S. Liang, R. Schneiter, D. Singleton, J. Wisniewska, and A.M. Tartakoff. 1994. Isolation and characterization of *Saccharomyces cerevisiae* mRNA transport-defective (mtr) mutants. *J. Cell Biol.* 126:649–659.

Kraemer, D.M., C. Strambio-de-Castillia, G. Blobel, and M.P. Rout. 1995. The essential yeast nucleoporin NUP159 is located on the cytoplasmic side of the nuclear pore complex and serves in karyopherin-mediated binding of transport substrate. *J. Biol. Chem.* 270:19017–19021.

Kutay, U., F.R. Bischoff, S. Kostka, R. Kraft, and D. Görlich. 1997. Export of importin α from the nucleus is mediated by a specific nuclear transport factor. *Cell*. 90:1061–1071.

Lin-Marq, N., and S.G. Clarkson. 1995. A yeast RNA binding protein that resembles the human autoantigen La. *J. Mol. Biol.* 245:81–85.

Makkerh, J.P.S., C. Dingwall, and R.A. Laskey. 1996. Comparative mutagenesis of nuclear localization signals reveals the importance of neutral and acidic amino acids. *Curr. Biol.* 6:1025–1027.

Meerovitch, K., Y.V. Svitkin, H.S. Lee, F. Lejbkowicz, D.J. Kenan, E.K. Chan, V.I. Agol, J.D. Keene, and N. Sonenberg. 1993. La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. *J. Virol.* 67:3798–3807.

Michaud, N., and D. Goldfarb. 1992. Microinjected U snRNAs are imported to oocyte nuclei via the nuclear pore complex by three distinguishable targeting pathways. *J. Cell Biol* . 116:851–861.

Michaud, N., and D.S. Goldfarb. 1991. Multiple pathways in nuclear transport: the import of U2 snRNP occurs by a novel kinetic pathway. *J. Cell Biol.* 112: 215–223.

Moritz, M., A.G. Paulovich, Y.-F. Tsay, and J.L. Woolford, Jr. 1990. Depletion of yeast ribosomal proteins L16 or rp59 disrupts ribosome assembly. *J. Cell Biol.* 111:2261–2274.

Moritz, M., B.A. Pulaski, and J.L. Woolford, Jr. 1991. Assembly of 60S ribosomal subunits is perturbed in temperature-sensitive yeast mutants defective in ribosomal protein L16. *Mol. Cell Biol.* 11:5681–5692.

Moroianu, J. 1997. Molecular mechanisms of nuclear protein transport. *Crit. Rev. Eukaryotic Gene Expr*. 7:61–72.

Moroianu, J., G. Blobel, and A. Radu. 1996. The binding site of karyopherin α for karyopherin β overlaps with a nuclear localization sequence. *Proc. Natl. Acad. Sci. USA*. 93:6572–6576.

Moroianu, J., G. Blobel, and A. Radu. 1997. RanGTP-mediated nuclear export of karyopherin α involves its interaction with the nucleoporin Nup153. *Proc. Natl. Acad. Sci. USA*. 94:9699–9704.

Nehrbass, U., and G. Blobel. 1996. Role of the nuclear transport factor p10 in nuclear import. *Science*. 272:120–122.

Nigg, E.A. 1997. Nucleocytoplasmic transport: signals, mechanisms and regulation. *Nature*. 386:779–787.

Rexach, M., and G. Blobel. 1995. Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell*. 83:683–692.

Rinke, J., and J.A. Steitz. 1982. Precursor molecules of both human 5S ribosomal RNA and transfer RNAs are bound by a cellular protein reactive with anti-La lupus antibodies. *Cell*. 29:149–159.

Rinke, J., and J.A. Steitz. 1985. Association of the lupus antigen La with a subset of U6 snRNA molecules. *Nucleic Acids Res*. 13:2617–2629.

Rose, M.D., F. Winston, and P. Hieter. 1990. Methods in Yeast Genetics: A Laboratory Course Manual. Cold Spring Harbor Press, Cold Spring Harbor, NY. 198 pp.

Rotenberg, M.O., M. Moritz, and J.L. Woolford, Jr. 1988. Depletion of *Saccharomyces cerevisiae* ribosomal protein L16 causes a decrease in 60S ribosomal subunits and formation of half-mer polyribosomes. *Genes Dev*. 2:160–172.

Rout, M.P., G. Blobel, and J.D. Aitchison. 1997. A distinct nuclear import pathway used by ribosomal proteins. *Cell*. 89:715–725.

Seedorf, M., and P.A. Silver. 1997. Importin/karyopherin protein family members required for mRNA export from the nucleus. *Proc. Natl. Acad. Sci. USA*. 94:8590–8595.

Shulga, N., P. Roberts, Z. Gu, L. Spitz, M.M. Tabb, M. Nomura, and D.S. Goldfarb. 1996. In vivo nuclear transport kinetics in *Saccharomyces cerevisiae*: a role for heat shock protein 70 during targeting and translocation. *J. Cell Biol.* 135:329–339.

Simons, F.H., F.J. Broers, W.J. Van Venrooij, and G.J. Pruijn. 1996. Characterization of cis-acting signals for nuclear import and retention of the La (SS-B) autoantigen. *Exp. Cell Res*. 224:224–236.

Stade, K., C.S. Ford, C. Guthrie, and K. Weis. 1997. Exportin 1 (Crm1) is an essential nuclear export factor. *Cell*. 90:1041–1050.

Stefano, J.E. 1984. Purified lupus antigen La recognizes an oligouridylate stretch common to the 3′ termini of RNA polymerase III transcripts. *Cell*. 36:145–154.

Tarn, W.-Y., T.A. Yario, and J.A. Steitz. 1995. U12 snRNA in vertebrates: evolutionary conservation of 5′ sequences implicated in splicing of pre-mRNAs containing a minor class of intron. *RNA*. 1:644–656.

Ullman, K.S., M.A. Powers, and D.J. Forbes. 1997. Nuclear export receptors: from importin to exportin. *Cell*. 90:967–970.

Yaffe, M.P., and G. Schatz. 1984. Two nuclear mutations that block mitochondrial protein import. *Proc. Natl. Acad. Sci. USA*. 81:4819–4823.

Yoo, C.J., and S.L. Wolin. 1994. La proteins from *Drosophila melanogaster* and *Saccharomyces cerevisiae*: a yeast homolog of the La autoantigen is dispensable for growth. *Mol. Cell Biol.* 14:5412–5424.

Yoo, C.J., and S.L. Wolin. 1997. The yeast La protein is required for the 3′ endonucleolytic cleavage that matures tRNA precursors. *Cell*. 89:393–402.

Zasloff, M. 1983. tRNA transport from the nucleus in a eukaryotic cell: carrier-mediated translocation process. *Proc. Natl. Acad. Sci. USA*. 80:6436–6440.