The Vertebrate GLFG Nucleoporin, Nup98, Is an Essential Component of Multiple RNA Export Pathways

Maureen A. Powers,* Douglass J. Forbes,* James E. Dahlberg,‡ and Elsebet Lund‡

*Department of Biology, University of California at San Diego, La Jolla, California 92093-0347; and ‡Department of Biomolecular Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Abstract. The 97-kD O-linked glycoprotein, Nup98, is a component of the Xenopus laevis nuclear pore complex and the only vertebrate GLFG nucleoporin identified (Powers, M.A., C. Macauley, F. Masiarz, and D.J. Forbes. 1995. J. Cell Biol. 128:721–736). We have investigated possible roles of xNup98 in the nucleocytoplasmic transport of proteins and RNAs by analyzing the consequences of injecting monospecific polyclonal antibodies to xNup98 into X. laevis oocytes. We show here that nuclear injection of anti-xNup98 inhibited the export of multiple classes of RNAs, including snRNAs, 5S RNA, large ribosomal RNAs, and mRNA. In contrast, the export of tRNA was unaffected. Injection of anti-xNup98 into the oocyte cytoplasm had no effect on export of any of the RNAs. Significantly, nuclear injection of anti-xNup98 antibodies did not inhibit import of either karyophilic proteins or snRNPs. This latter result is in agreement with our previous finding that Nup98 is not an essential element of the protein import pathway. Thus, Nup98 plays a role specifically in RNA export from the nucleus, and it appears to be an essential component of multiple RNA export pathways.

Traficking across the nuclear envelope occurs exclusively through the nuclear pore complex, which both imports proteins and small nuclear ribonucleoproteins (snRNPs)¹ and exports RNAs and ribosomal subunits. In addition to the proteins of the pore, nucleocytoplasmic transport requires soluble factors such as the importin α/β heterodimer, which binds directly to nuclear-targeted proteins, and the GTPase, Ran, with its associated stimulatory and recycling factors (for review see Moore and Blobel, 1994; Powers and Forbes, 1994; Melchior and Gerace, 1995; Görlich and Mattaj, 1996; Sazer, 1996).

The nuclear pore complex itself is a large and elaborate structure of 120 MD in vertebrates, comprising ~100 different proteins, many of which are present in multiple copies (for review see Rout and Wente, 1994; Davis, 1995). Structurally, the pore consists of a core of eight spokes surrounding a central transporter which spans the nuclear envelope. This core structure is flanked by a cytoplasmic ring, from which fibers project into the cytoplasm, as well as a nuclear ring from which a basket-like structure extends into the nucleoplasm (for review see Pante and Aebi, 1993; Rout and Wente, 1994). Additional long fibers project from the basket into the nucleus (Cordes et al., 1993). Both the cytoplasmic fibers and the nuclear basket have been hypothesized to play roles in the initial binding of transport substrates to the pore. Indeed, scanning electron microscopy of Balbiani ring transcripts shows movement through the basket (Kiseleva et al., 1996).

Much progress has been made recently in our knowledge of the nuclear pore complex. In yeast, multiple nucleoporin genes have been identified, and mutational analysis has linked functional or structural phenotypes with specific gene products (for review see Doye and Hurt, 1995). In vertebrates, 12 of the potential ~100 nucleoporins have been identified and localized to specific substructures of the pore (for review see Pante and Aebi, 1993). Of these 12, approximately half contain repeated peptide motifs: FXFG in the majority (for review see Fabre and Hurt, 1994; Davis, 1995), and GLFG in a single protein, Nup98 (Powers et al., 1995; Radu et al., 1995b). Several lines of evidence implicate the FXFG family of nucleoporins as crucial to nuclear transport. In vivo, antibodies to the FXFG family impair both nuclear protein import and RNA export (Dabauvalle et al., 1988; Featherstone et al., 1988; Terns and Dahlberg, 1994; Lund and Dahlberg, manuscript in preparation). Recently, a member of this family was reported to play a role in the export of polyA⁺ RNA (Bastos et al., 1996). FG repeat-containing nucleoporins of the cytoplasmic fibers (Nup 358 and Nup214) and the nuclear basket (Nup153 and Nup98) have been shown to bind soluble transport factors in vitro (Moroianu et al., 1995; Radu et al., 1995a; Radu et al., 1995b). Moreover,
binding experiments using isolated yeast nucleoporins suggest that interactions between such repeat domains and import factors may be functionally important in transport (Rexach and Blobel, 1995; Nehrbass and Blobel, 1996). Finally, in vitro nuclear reconstitution has indicated the importance of FXFG nucleoporins in nuclear import (Dabauvalle et al., 1990; Finlay and Forbes, 1990; Finlay et al., 1991; Miller and Hanover, 1994).

At present, Nup98 is the only vertebrate nucleoporin identified that contains GLFG repeats. Interestingly, reconstituted nuclei that lack this GLFG nucleoporin are competent for nuclear protein import. However, xNup98-depleted nuclei remain small and fail to replicate their DNA, indicating an essential nuclear role for Nup98, but not one in import (Powers et al., 1995). Immunofluorescence shows that xNup98 is present both at the pore and within the nuclear interior (Powers et al., 1995), and rat Nup98 has been localized within the pore to the nuclear basket by immunoelectron microscopy (Radu et al., 1995b). In vitro binding assays indicate that rat Nup98 can interact with soluble cytoplasmic import factors (Moradian et al., 1995; Radu et al., 1995b). However, given the localization of Nup98 at the nuclear basket and within the nucleus, the functional significance of this latter interaction is not understood.

The GLFG family in the yeast Saccharomyces cerevisiae includes five nucleoporins: Nup49, Nup54, Nup100, Nup116, and Nup145 (Wente et al., 1992; Wimmer et al., 1992). Mutations in members of this family have pleiotropic effects on yeast nuclear function, including aberrant nuclear envelope structure, nuclear accumulation of polyA+ RNA, and impaired nuclear import (for review see Doyle and Hurt, 1995). Nup49 and Nup54 are essential proteins present in a multiprotein complex that is primarily required for nuclear protein import (Schlenstedt et al., 1993; Grandi et al., 1995). Deletion of the essential Nup145 gene results in a defect not in protein import, but in poly A+ RNA export (Fabre et al., 1994). Nup100, Nup116, and Nup145 each contains a related domain that can bind homopolymeric RNA in vitro (Fabre et al., 1994). A similar domain is found in rat Nup98, which shows strong homology to this subset of the GLFG family (Radu et al., 1995b); peptide analysis of Xenopus Nup98 indicates that this domain is conserved in Xenopus (Powers et al., 1995). In yeast, the presence of a single gene containing this putative RNA-binding domain is sufficient for cell viability; thus Nup145, Nup116, and Nup100 appear to serve a redundant function, most likely in the export of RNA.

Export of different classes of RNA, including snRNAs, mRNA, tRNA, and ribosomal RNA, occurs via distinct pathways (for review see Izaurralde and Mattaj, 1995). This led to a model in which export of different RNAs is mediated by distinct and class-specific saturable factors. In support of this model, recent studies have identified several soluble RNA binding factors in vertebrates, such as the cap binding complex (CBC), TFIIIA, and the Rev protein of HIV-1, which bind to snRNAs, 5S RNA, and unspliced HIV RNA, respectively (Guddat et al., 1990; Izaurralde and Mattaj, 1992; Fischer et al., 1995; Izaurralde et al., 1995). It is thought that each may specifically facilitate the export of the bound RNA. In contrast, the components of the vertebrate nuclear pore that constitute the export machinery have remained, for the most part, uncharacterized.

Given the contributions of GLFG nucleoporins to nuclear export in yeast, we asked whether xNup98 might be an essential component of the vertebrate RNA export machinery. For this, we have used Xenopus laevis oocytes, which allow for microinjection of transport substrates and potentially inhibitory antibodies into either the nuclear or cytoplasmic compartment. We find that affinity purified antibodies to Xenopus Nup98, when injected into oocyte nuclei, selectively inhibit the nuclear export of multiple, but not all, classes of RNAs. However, xNup98 antibodies do not significantly impair nuclear import of either snRNPs or karyophilic proteins. These results argue strongly that Xenopus Nup98 functions as a common element in multiple pathways of RNA export from the nucleus, but not as an essential component of nuclear import pathways.

Materials and Methods

DNA Templates for In Vivo and In Vitro RNA Synthesis

Templates for in vitro RNA synthesis in oocytes were plasmid DNAs containing genes for X. laevis U4 snRNA (Vankan et al., 1990), 5S rRNA (Wolfle et al., 1986), and tRNA5′ (Gouliloud and Clarkson, 1986). Templates for in vitro RNA synthesis were DNA fragments generated by PCR amplification, as described previously for U1α, U1β, U6 snRNAs (Terns et al., 1993a), U5 snRNA (Pasquinelli et al., 1995), and Nl15 RNA (Grimm et al., 1997). The template for AdML pre-mRNA was Smα1-linearized pSP64-Ad1(+/−A) plasmid DNA (Lund and Dahlberg, manuscript in preparation). pSP64-Ad1(+/−A) contains the Pst1-Saula1A fragment of pBSAd1 (Konarska and Sharp, 1987) cloned between the Pst1 and BamHI sites of pSP64 Poly(A) (Promega Corp., Madison, WI).

Antibodies for Microinjection or Immunoprecipitation

Affinity purified polyclonal rabbit antibodies to the Xenopus nucleoporins xNup98 and xNup200 were prepared as previously described (Macaulay et al., 1995; Powers et al., 1995). To generate preparations suitable for oocyte injection, affinity purified antibodies were further concentrated to 0.5–1 mg immunoglobulin/ml using a microconcentrator (Microcon 50; Amicon Corp., Danvers, MA). Immunoblots were performed as described previously, using peroxidase-conjugated secondary antibodies and a chemiluminescent substrate (Powers et al., 1995). Crude sera containing the polyclonal rabbit anti-mG antibodies (Munns et al., 1982) used for immunoprecipitation of precursor snRNAs were a generous gift of T. Munns (Washington University School of Medicine, St. Louis, MO).

Oocyte Injection and Analysis of 32P-labeled RNAs Made In Vivo

Stage V and VI oocytes were obtained from X. laevis frogs as described (Lund and Dahlberg, 1989). For in vivo synthesis of RNAs, 12–15 nl of a solution containing a mixture of plasmid DNAs (Fig. 1, legend) plus blue dextran (as a marker) was injected into the oocyte nucleus. To monitor synthesis of the RNAs, 10 μCi of 32P-labeled GTP (NEN-Dupont, Boston, MA) was injected into the cytoplasm of each oocyte, and the intracellular distribution of the newly made transcripts was assayed with time. When used, control IgG and anti-nucleoporin antibodies were either coinjected with the DNA into the nucleus (6–15 ng/oocyte) or injected separately into the cytoplasm (15–30 ng/oocyte) prior to the cytoplasmic injection of labeled GTP. Nuclei and cytoplasm were isolated from successfully in-
Nup98 is required for nuclear export of most RNAs.

### Analysis of Protein Import

Deproteinized nuclear RNAs were immunoprecipitated with anti-mG antibodies bound to protein A-Sepharose (Sigma Chemical Co., St. Louis, MO) as previously described (Neuman de Veygarg, Dahlberg, 1990; Terns et al., 1993b). The RNAs in both precipitate and supernatant fractions were analyzed by PAGE, and the levels of mG-capped snRNA precursors were quantitated by PhosphorImager analysis.

### Immunoprecipitation of snRNA Precursors

In vitro transcription with SP6 RNA polymerase (for AdML pre-mRNA and U1snRNA, U1g, and U5 snRNAs) or T7 RNA polymerase (for U6 snRNA and U15 RNA) was done as described previously (Melton et al., 1984; Pasquinelli et al., 1995) using α-[32P]GTP as the label. AdML pre-mRNA, U1snRNA, U1g, and U5 snRNA transcripts were synthesized with mGpppG caps, and U6 snRNA was synthesized with a γ-mpppG cap (Terns et al., 1993a). All RNA products were purified by electrophoresis in 8% denaturing polyacrylamide gels. For use in transport assays, 12–15 nl of a mixture of RNAs (1–10 fmol each, see Figs. 2 and 4, legends) was injected into the nucleus or cytoplasm of oocytes. For nuclear injections, U6 snRNA, which is not exported to the cytoplasm (Vankan et al., 1990; Terns et al., 1993a), and blue dextran (Jarmolowski et al., 1994) was included as controls for the accuracy of injections and dissections. After different times of incubation, the intracellular distributions of the injected RNAs were analyzed as described above for in vivo transcribed RNAs.

### In Vivo Synthesis of 35S-labeled Nuclear Proteins and Analysis of Protein Import

To produce 35S-labeled Xenopus karyophilic proteins, 100–150 stage V and VI oocytes were incubated for 24 h in 1 ml of MBS-H medium (Gurdon and Wickens, 1983) containing 0.5–1.0 mCi of [35S]methionine (TRAN35S-LABEL™, ICN Radiochemicals, Irvine, CA). Labeled nuclei were isolated by manual dissection of oocytes under mineral oil (Lund and Paine, 1990) and collected in a 0.5-ml microfuge tube on ice. After removal of excess mineral oil, 50–100 nuclei were homogenized in 10–20 μl of protein buffer (10 mM MOPS, pH 7.2, 75 mM KCl, 25 mM NaCl containing 1 mM DTT, and 1 μg/ml each of aprotinin, leupeptin, and pepstatin) by repeated pipetting. The homogenate was centrifuged at 14,000 rpm for 4 min in a microfuge; the clarified supernatant was collected and either used immediately or stored at −70°C for later injection. For protein import assays, 35–50 nl of 35S-labeled nuclear extract (~0.25 oocyte equivalents) was injected into each oocyte cytoplasm. Where used, control IgG and either anti-nucleoporin antibodies or wheat germ agglutinin (WGA; Vector Labs, Inc., Burlingame, CA) was injected separately into the nuclei or cytoplasm before cytoplasmic injection of the import substrate. For WGA, 8–10 nl or 15–30 nl of a saturated solution (~20 mg/ml in 88 mM NaCl, 10 mM TrisHCl, pH 8.0) was injected per nucleus or cytoplasm, respectively. After overnight incubation, oocytes (at least 5/sample) were manually dissected, and the intracellular distribution of labeled proteins was determined by analysis of 0.5–1.0 oocyte equivalents of the nuclear and cytoplasmic fractions in 10% polyacrylamide gels containing SDS (Adolph et al., 1977). Autoradiograms of the dried gels were exposed for 2–14 d at room temperature.

### Results

**Anti-xNup98 Antibodies Inhibit Nuclear Export of snRNA and 5S RNA, but Not tRNA**

Previously, we showed that the Xenopus nucleoporin xNup98, which is located both in the nuclear interior and at the nucleoplasmic side of the nuclear pore, is not essential for protein import (Powers et al., 1995). To determine if Nup98 is required for transport out of the nucleus, we examined the effect of anti-xNup98 antibodies on the nuclear export of different classes of RNA.

Anti-xNup98 antibodies, prepared and affinity purified, as previously described, recognized a single WGA binding protein from Xenopus egg extracts (Fig. 1 A, lane 3; Powers et al., 1995). This antibody also specifically recognized a single protein of identical size in the enriched nuclear pores of Xenopus annulate lamellae, indicating that the antibody does not cross-react with any other component of the pore (Fig. 1 A, lane 4; Meier et al., 1995). In addition, the antibody was seen to cross-react with both the GLFG repeat and RNA binding domains of the homologous rat Nup98 (Fig. 1 B, lanes 2–4). A nonspecific rabbit IgG as well as antibodies to the Xenopus nucleoporin p200, which is the homolog of the mammalian nucleoporin Nup214/CAN and is localized to the cytoplasmic face of the pore (Miller and Hanover, 1994; Kraemer et al., 1994; Macaulay et al., 1995), were used for control antibody injections.

To examine their effect on RNA export, xNup98 specific antibodies were injected into Xenopus oocyte nuclei together with a mixture of plasmid DNAs encoding Xenopus U4 snRNA, 5S rRNA, and tRNA Tyr (Fig. 2, α-98). The

![Figure 1. Specificity of affinity-purified anti-xNup98 antibodies.](image-url)
DNA templates were also injected with either buffer alone (Fig. 2, Control) or with affinity purified antibodies against the Xenopus nuclear pore protein, Nup200 (α-200). Anti-xNup200 antibodies injected into the nucleus have no access to their cytoplasmic antigen and serve as controls for nonspecific effects of antibody injection. After a short incubation to allow for the binding of the antibodies and formation of transcription complexes, [32P]GTP was injected into the oocyte cytoplasm. At various time points, oocytes were manually dissected into nuclear and cytoplasmic fractions, and the newly synthesized, labeled RNAs in each fraction were isolated and resolved by PAGE.

In sharp contrast to the control injections, injection of antibodies to xNup98 strongly inhibited the export of U4 snRNA from the oocyte nuclei (Fig. 2, A and B, lanes 3 and 4). At both the 4 and 21 h timepoints, the level of U4 RNA in the cytoplasm of oocytes injected with anti-xNup98 was greatly reduced relative to the level in oocytes injected with either buffer or the control antibody (Fig. 2, A and B; lanes 2, 4, and 6). This inhibition of export was apparent even at incubation times as short as 2 h (data not shown).

Normally, m7G-capped precursor snRNA is rapidly exported to the cytoplasm. There, it becomes complexed with cytoplasmic Sm proteins, and its cap is hypermethylated to the mature m2,2,7G form. The fully matured snRNP can then be imported back into the nucleus (Mattaj, 1986). It was thus important to determine whether the U4 RNA in the nuclei of anti-xNup98 injected oocytes had been exported to the cytoplasm and reimported, or had remained in the nucleus due to a block in export. To determine this, we immunoprecipitated nuclear RNAs with an antibody that specifically recognizes the m7G-cap common to RNA polymerase II transcripts (Munns et al., 1982). In control oocytes at both 4 and 21 h, the vast majority of U4 RNA in the nucleus consisted of the mature, nonprecipitable m2,2,7G-capped form, indicating it had been reimported (Fig. 3, lanes 1–3 and 7–9; Neuman de Vegvar and Dahlberg, 1990). In contrast, the U4 RNA present in nuclei of anti-xNup98 treated oocytes at 4 h was almost exclusively the m7G-capped, precursor forms (Fig. 3, lanes 4–6). Thus, export of newly synthesized snRNAs had been effectively blocked by microinjection of anti-xNup98 antibodies into the nucleus. Even at 21 h, only a small amount of mature U4 RNA had accumulated in the nuclei of anti-xNup98 treated oocytes (Fig. 3, lane 11), showing that the inhibitory effect on snRNA export was persistent. When anti-xNup98 antibodies were injected into the cytoplasm of oocytes, no deficiency in RNA transport was observed, consistent with the nuclear localization of the target Nup98 nucleoporin (data not shown). The reduced level of U4 snRNA in anti-xNup98 injected oocytes was not due to a defect in U4 transcription (data not shown) but to the inherent instability of m7G-capped precursor U4 snRNA, an instability also seen in other cases where pre-U4 is retained in the nucleus (Lund, E., and J.E. Dahlberg, manu-
The export of both 5S RNA and tRNA occurs by different pathways than U4 snRNA, as determined by competition studies (Jarmolowski et al., 1994). When the export of 5S rRNA was examined, it was found to be substantially reduced in the presence of nuclear xNup98 antibodies (Fig. 2, lanes 2, 4, and 6). A reduced amount of 5S DNA was injected for the 21-h timepoint to avoid overwhelming the signals from other transcripts (Fig. 2, legend). Strikingly, the export of tRNA was completely unaffected by anti-xNup98 antibodies at all times. The persistence of tRNA export in the presence of anti-xNup98 antibodies makes it unlikely that the inhibition of U4 and 5S RNA export resulted from physical obstruction of the nuclear pore. Instead, we conclude that the binding of antibody to xNup98 prevents a specific function of this protein, a function required for multiple pathways of RNA export.

Anti-xNup98 Antibodies Impair mRNA Export

Although both mRNAs and pre-snRNAs contain 5‘ m7G-caps which serve as signals for export from the nucleus (Hamm and Mattaj, 1990; Izaurralde et al., 1992; Terns et al., 1993a; Izaurralde et al., 1995), transport of these RNAs apparently occurs via different pathways (Jarmolowski et al., 1994; Fischer et al., 1995). To determine whether the export of mRNA is also dependent on Nup98 function, we monitored the export of an mRNA that was generated by splicing of injected 32P-labeled adenovirus major late (AdML) pre-mRNA in oocyte nuclei (Hamm and Mattaj, 1990; Lund and Dahlberg, manuscript in preparation). Oocytes were injected in the nucleus twice, first with either xNup98-specific antibodies or buffer, and then, after a short incubation to allow for antibody binding, with a mixture of the 32P-labeled pre-mRNA and control RNAs. As a control for the accuracy of the second nuclear injection, we included labeled U6 snRNA, which is not exported from the nucleus. As a further test for inhibition of snRNA export by anti-xNup98, we also included U1Sm–RNA, a mutant U1RNA which would normally be exported, but which lacks the Sm binding site required for import back into the nucleus (Hamm and Mattaj, 1990).

In control oocytes, the spliced AdML mRNA was ex-
ported in a time-dependent manner (Fig. 4, mRNA, lanes 3 and 4, 7 and 8). In contrast, in anti-xNup98 injected oocytes, the majority of spliced mRNA remained in the nucleus (Fig. 4, lanes 1 and 2, 5 and 6). The level of nuclear mRNA at 4.5 h in anti-xNup98 injected oocytes (Fig. 4, lane 5) was reduced relative to the level at 1.5 h (Fig. 4, lane 7), due to the RNA turnover that occurs in the nucleus when export is inhibited; still, the majority of the mRNA observed was nuclear, not cytoplasmic (Fig. 4, lanes 5 and 6).

U1smRNA also was exported to the cytoplasm in control oocytes, but was largely retained in the nucleus in anti-xNup98 injected oocytes. Control U6 snRNA and the lariat of the pre-mRNA, RNAs normally not exported, stayed localized in the nucleus in both control and anti-xNup98 injected oocytes. A certain amount of unspliced pre-mRNA export was observed in control injected oocytes, but was largely retained in the nucleus in anti-xNup98 injected oocytes. A certain amount of unspliced pre-mRNA export was observed in control injected oocytes, most likely due to initial saturation of the splicing machinery (Lund and Dahlberg, unpublished results). Strikingly, however, this export was also sensitive to inhibition by anti-xNup98 antibodies.

In parallel experiments, we found that the simultaneous injection of anti-xNup98 antibodies with snRNA and pre-mRNA resulted in little or no inhibition of export (data not shown). The necessity for preinjection to allow binding of antibody to antigen supports our conclusion that inhibition of RNA export results from specific interaction of the antibodies with xNup98. Taken together, the results of Figs. 2, 3, and 4 indicate that both mRNA and snRNA transport is inhibited; still, the majority of the mRNA observed was nuclear, not cytoplasmic (Fig. 4, lanes 5 and 6).

**Anti-xNup98 Antibodies Decrease Ribosomal RNA Export**

We next asked whether xNup98 function was also required for export of the large 18 and 28S ribosomal RNAs, which occurs by yet another distinct RNA export pathway (Bataillé et al., 1990; Fischer et al., 1995; Pokrywka and Goldfarb, 1995). In *Xenopus* oocytes, the rRNA genes are highly amplified, so it is possible to monitor expression of the endogenous RNA genes simply by injection of ([³²P]GTP (Peculis and Steitz, 1993). We found that anti-xNup98 antibodies significantly retarded export of the large 18 and 28S ribosomal RNAs, as shown by the reduction in levels of these newly made rRNAs in the cytoplasm (Fig. 5, lanes 4 and 5). The concomitant accumulation of excess mature 18 and 28S rRNAs in the nucleus (Fig. 5, lane 2) showed that anti-xNup98 antibodies inhibited export but not the synthesis or maturation of ribosomal RNAs. We also note that export of rRNAs, in the form of large preribosomal subunits, is inhibited to a lesser extent than is the export of the much smaller 5S RNA particles. This is a further indication that inhibition does not result simply from nonspecific obstruction of the nuclear pore. Additionally, maturation of the nascent labeled rRNA transcripts requires the assembly of the ribosomal subunits in the nucleus which, in turn, depends on the import of ribosomal proteins (for review see Hadjiolov, 1985). Thus, the results in Fig. 5 suggested that protein import is not influenced by the anti-xNup98 antibodies.

**Anti-xNup98 Antibodies Have No Effect on Nuclear Protein Import**

We have previously shown that xNup98 is not required for nuclear protein import in reconstituted nuclei (Powers et al., 1995). To test whether Nup98 is required for import in vivo, we compared the nuclear import of karyophilic proteins in oocytes pre-injected with anti-xNup98 antibodies, control IgG antibodies, or WGA, a potent inhibitor of protein import (Forbes, 1992). After antibody injection, total [³⁵S]-labeled nuclear proteins (Dabauvalle et al., 1988) were injected into the cytoplasm of the oocytes. 24 h later, the oocytes were dissected into nuclear and cytoplasmic fractions for determination of the intracellular distribution of labeled proteins. Injection of anti-xNup98 antibodies into the nucleus had no detectable effect on nuclear import, as shown by the efficient uptake of the major labeled nuclear proteins, N1/N2, as well as other nuclear proteins (Fig. 6, lanes 2 and 4). Cytoplasmic injection of anti-xNup98 also had no effect on nuclear protein import (data not shown). As expected, the import of proteins containing nuclear localization signals (NLS) was strongly inhibited in oocytes that were injected with WGA (Fig. 6, lanes 5 and 6; Finlay et al., 1987; Dabauvalle et al., 1988; Fischer et al., 1991).
Thus, antibodies to xNup98 do not act as a physical block to protein transit through the nuclear pore, but rather, define a specific and essential role for this nucleoporin in RNA export.

**Anti-xNup98 Antibodies Do Not Prevent snRNP Import**

Certain small RNAs, like U6 snRNA (Vankan et al., 1990; Terns et al., 1993a; Boelens et al., 1995) and the synthetic NL-15 RNA (Grimm et al., 1997), are not normally exported from the nucleus. However, if injected into the cytoplasm, these RNAs can be imported into the nucleus via the same WGA-sensitive pathway as NLS-containing proteins (Fischer et al., 1991; Grimm et al., 1997). Other RNAs, like U1 and U5 snRNAs, which normally undergo maturation in the cytoplasm, are imported via a separate, snRNP-specific pathway (Mattaj, 1986) that is less sensitive to WGA inhibition (Fischer et al., 1991; Michaud and Goldfarb, 1991; Lund and Dahlberg, manuscript in preparation). We found that nuclear import of cytoplasmically injected NL-15 RNA was completely unaffected by the presence of anti-xNup98 antibodies (Fig. 7), in agreement with the results for protein import (Fig. 6). Likewise, anti-xNup98 antibodies had little if any effect on the import of U5 or U1 snRNAs via the snRNP pathway (Fig. 7, lanes 2 and 3). In contrast, WGA completely blocked NL15 import and impaired U1 and U5 import (Fig. 7, lane 4). Taken together, these results strongly argue that Nup98 is not essential for nuclear import of RNA.

**Discussion**

By monitoring nuclear transport in *Xenopus* oocytes, we have demonstrated that this GLFG nucleoporin functions in the export of multiple classes of RNA, but not in the export of tRNA. Further, our results show that Nup98 does not have an essential role in nuclear protein import, supporting our previous conclusion from nuclear reconstitution studies. Similarly, we find that Nup98 is not required for the import of spliceosomal snRNPs. Thus, Nup98 represents the first vertebrate nuclear pore component for which a unidirectional role in multiple RNA export pathways has been established.

The existence of distinct pathways of nuclear export for different classes of RNA is suggested both by kinetic analyses (Zasloff, 1983; Jarmolowski et al., 1994; Lund and Dahlberg, manuscript in preparation) and by competition experiments, which demonstrate that various RNAs (e.g., snRNA, mRNA, and tRNA) are unable to compete with one another for export (Jarmolowski et al., 1994; Boelens et al., 1995; Pokrywka and Goldfarb, 1995; Simons et al., 1996). These latter results indicate the existence of at least one specific limiting factor in the export of each class of RNA. Since it is believed that RNA exits the nucleus as an RNA–protein complex, it is likely that specific RNA binding proteins represent these limiting factors, and indeed candidate proteins have been identified. TFIIIA, which binds to 5S RNA, contains a nuclear export signal (NES) that may mediate interaction with the export machinery (Guddat et al., 1990; Fridell et al., 1996). CBC, which binds to m7G-capped RNAs, is thought to promote export of snRNA precursors (Izaurralde et al., 1992). Antibodies to the CBP20 protein, a CBC subunit, are capable of reducing the export of snRNA (Izaurralde et al., 1995). The CBC complex may also be involved in mRNA export (Visa et al., 1996), although antibodies to CBP20 have no effect on mRNA export (Izaurralde et al., 1995). The hnRNP A1 protein may fulfill the role of a class-specific export factor for mRNA, since this protein binds to mRNA, shuttles between nucleus and cytoplasm, and contains a unique NES sequence (Michael et al., 1995). The retrovirus HIV-1 encodes its own specific export factor, the Rev protein, which recognizes a sequence present only in incompletely spliced viral RNAs (for review see Hope and Pomerantz, 1995) and, via an NES sequence, mediates the export of these RNAs to the cytoplasm (Fischer et al., 1995). No class-specific factor has yet been identified for tRNA.

The next question is what then recognizes the different class-specific export factors. One possibility is that a general export receptor recognizes NES signals present in the individual class-specific factors and ferries the RNA–protein complexes to the pore. Alternatively, different RNAs, via their class-specific factors, may bind directly to a protein or proteins of the nuclear pore. Nup98, localized both on the nucleoplasmic side of the pore and within the nucleus, potentially on intranuclear filaments, is ideally positioned to mediate either the binding of a general export receptor, or the binding of individual class-specific factors. Antibodies to xNup98 impair snRNA, mRNA, 5S RNA, and ribosomal RNA export, indicating involvement of this nucleoporin in all of these pathways (Figs. 2, 4, and 5). The saturability of tRNA export indicates that this class of RNA also has a specific export system (Zasloff, 1983), but the lack of inhibition by anti-xNup98 antibodies (Fig. 2) implies that such export bypasses the need for Nup98. Thus, the export of tRNA might involve binding to a different nucleoporin, or tRNA may enter the export ma-
chinery at a point downstream of Nup98. Interestingly, we have previously found that tRNA export, unlike that of most other RNAs, occurs independently of the Ran GTPase cycle (Cheng et al., 1995).

All classes of RNA export, including tRNA export, can be blocked by certain inhibitors; however, because these compounds also block protein import, it is unclear if they affect RNA export directly. For example, injection of the lectin WGA inhibits export of all classes of RNA (Featherstone et al., 1988; Batalilé et al., 1990; Neuman de Vega and Dahlberg, 1990), but it binds to N-acetylglucosamine residues present on multiple glycoproteins of the nuclear pore (Nup200, Nup98, and p62 in *Xenopus*; Forbes, 1992), making conclusions about the role of any given nucleoporin difficult to interpret. Similarly, nuclear injection of the monoclonal antibody, 414, which binds to the FXFG repeat sequences found in some nucleoporins (Nup200, Nup153, and p62), blocks numerous classes of RNA export but not that of tRNA (Terns and Dahlberg, 1994; Lund and Dahlberg, manuscript in preparation). However, because this antibody recognizes both Nup153 on the basket and p62 in or near the central transporter (Pante and Aebl, 1993), its inhibition of export can not be attributed to any specific nucleoporin. Both these reagents, WGA and mAb 414, also block the import of proteins into the nucleus (Finlay et al., 1987; Featherstone et al., 1988; Lund and Dahlberg, manuscript in preparation). Thus, only now in the case of Nup98 has a monospecific reagent implicated a single vertebrate nucleoporin in a specific transport process, RNA export.

If a general export receptor carries the different RNAs to the pore, what might that export receptor be? It is presently not known whether either of the components of the heterodimeric import receptor, importins α and β, might play a role in export through the pore. In nuclear import, importin α binds to the NLS of a nuclear-targeted protein; importin β then acts to dock the import receptor complex to proteins of the nuclear pore (for review see Melchior and Gerace, 1995; Görlich and Mattaj, 1996). The observations that importin β can bind to numerous repeat-containing nucleoporins on blots, including Nup98, led to a proposal that Nup98 has a role in nuclear protein import (Moroianu et al., 1995; Radu et al., 1995b). However, in our earlier studies, we found that nuclei reconstituted in the absence of Nup98 were competent for protein import, indicating that binding of importin β to Nup98 is not essential for this process (Powers et al., 1995). Consistent with this, we find here that anti-xNup98 antibodies inhibit export but have no significant effect on import of either proteins or snRNPs. Thus, while there may be an interaction between Nup98 and importin β, we have found no functional evidence supporting a role for Nup98 in nuclear import.

If importin β has a function in RNA export, it might be mediated through the interaction of this factor with the GLFG domain of Nup98 (Radu et al., 1995b). Indeed, in yeast, importin β interacts with the GLFG domain of Nup116, a homolog of Nup98, in a two hybrid assay. Moreover, the GLFG domain of Nup116 is the only GLFG domain of any yeast nucleoporin essential for cell viability, and overexpression of this domain in yeast results in loss of poly A+ RNA export (Iovine et al., 1995). However, in a separate study using recombinant subdomains of nucleoporins, yeast importin β was found to interact with the yeast Nup1 FXFG domain but not with the GLFG domains of either Nup145 or Nup54 (Nup116 was not tested in this assay; Rexach and Blobel, 1995). These conflicting results raise questions about the specificity of interactions between purified, recombinant proteins in vitro and emphasize the importance of conducting parallel functional assays, as was done with Nup116 (Iovine et al., 1995). During protein import, vertebrate importin β traverses the pore but remains bound to the nuclear side of the pore and is not released into the nucleoplasm (Görlich et al., 1995). One possibility is that the GLFG domain of Nup98 may provide a final docking site for this import factor before it is recycled to the cytoplasm, thus explaining the interaction of Nup98 with importin β.

Several other regions of Nup98 present interesting possibilities for a potential function in export. A distinct sequence of 40 amino acids devoid of any repeat motifs is present near the NH2 terminus of Nup98, within the GLFG repeat domain (amino acids 170–192; Radu et al., 1995b). A segment of this region, rich in charged residues, is identical in *Xenopus* and rat Nup98, highly conserved in yeast Nup116, and absent from other members of the GLFG family (Nup49, Nup54, Nup100, and Nup145; Wente et al., 1992; Wimmer et al., 1992; Fabre et al., 1994; Wente and Blobel, 1994). In Nup116, a somewhat larger region containing this sequence is essential for function, as indicated by the loss of cell viability when it is deleted (Iovine et al., 1995). Because of its strong evolutionary conservation, this small domain may well be important for the RNA export function of Nup98.

Alternatively, the COOH-terminal domain of Nup98 contains a sequence of ~150 amino acids; this domain has no GLFG repeats but exhibits weak homology to certain RNA binding proteins (amino acids 737–892; Radu et al., 1995b). A homologous region is present in the yeast nucleoporins Nup100, Nup116, and Nup145, but presumably serves a redundant function in the three yeast nucleoporins, since the presence of any one copy of the sequence is sufficient for cell viability (Fabre et al., 1994). This sequence may be involved in RNA export, but in no case has the defect in yeast poly A+ RNA export been linked directly to deletion of this putative RNA-binding sequence. In vitro, the region was found to bind preferentially to poly rG homopolymers (Fabre et al., 1994). Under similar conditions, we find that Nup98 from *Xenopus* egg extracts also exhibits preferential binding to poly rG over other homopolymers (Powers and Forbes, unpublished results). However, as was the case for the yeast GLFG proteins (Fabre et al., 1994), only a very small fraction of the input xNup98 bound to the homopolymer. Since many other proteins of the egg extract also bind preferentially to poly rG, the significance of such in vitro interactions should be interpreted with caution. When poly rG and poly rI are injected into oocyte nuclei, inhibition of RNA export has been observed (Jarmolowski et al., 1994); but it remains to be determined whether such inhibition of export is due to binding and inhibition of Nup98 or to inhibition of another component of the RNA export machinery. Because our polyclonal antibody strongly interacts with both the GLFG and RNA-binding domains of Nup98 (Fig. 1B), we can not
precisely distinguish whether both or only one domain is involved in RNA export. It will be interesting to test even more specific antibody tools as they are developed.

In summary, we have demonstrated that the GLFG nucleoporin Nup98 plays a central role in the export of multiple classes of RNAs. The future identification of the nucleoporin factors that specifically interact with Nup98 and Nup153 (Bastos et al., 1996), either alone or complexed with an RNA export substrate, will be important for understanding the role of this and other nucleoporins in RNA export.

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