Cytoplasmic Transport of Ribosomal Subunits Microinjected into the Xenopus laevis Oocyte Nucleus: A Generalized, Facilitated Process

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Abstract. To study the biochemistry of ribonucleoprotein export from the nucleus, we characterized an in vivo assay in which the cytoplasmic appearance of radiolabeled ribosomal subunits was monitored after their microinjection into Xenopus oocyte nuclei. Denaturing gel electrophoresis and sucrose density gradient sedimentation demonstrated that injected subunits were transported intact. Consistent with the usual subcellular distribution of ribosomes, transport was unidirectional, as subunits injected into the cytoplasm did not enter the nucleus. Transport displayed properties characteristic of a facilitated, energy-dependent process; the rate of export was saturable and transport was completely inhibited either by lowering the temperature or by depleting nuclei of ATP; the effect of lowered temperature was completely reversible. Transport of injected subunits was likely a process associated with the nuclear pore complex, since export was also inhibited by prior or simultaneous injection of wheat germ agglutinin, a lectin known to inhibit active nuclear transport by binding to N-acetyl glucosamine–containing glycoproteins present in the NPC (Hart, G. W., R. S. Haltiwanger, G. D. Holt, and W. G. Kelly. 1989. *Annu. Rev. Biochem.* 58:841-874). Although GlcNAc modified proteins exist on both the nuclear and cytoplasmic sides of the nuclear pore complex, ribosomal subunit export was inhibited only when wheat germ agglutinin was injected into the nucleus. Finally, we found that ribosomal subunits from yeast and Escherichia coli were efficiently exported from Xenopus oocyte nuclei, suggesting that export of some RNP complexes may be directed by a collective biochemical property rather than by specific macromolecular primary sequences or structures.

THE assembly of ribosomes in eukaryotic cells is a process that requires transfer of macromolecules into and out of the nucleus (33). Ribosomal proteins, synthesized in the cytoplasm, enter the nucleus and assemble with nascent rRNA to form a preribosomal particle. Through a series of maturation steps involving endonucleolytic cleavage of the primary rRNA transcript and further addition of specific ribosomal proteins, the preribosomal particle splits into partially completed 40S and 60S subunits that contain the nearly mature 18S and 28S:5.8S rRNAs, respectively. The pre-60S subunit also acquires a 5S rRNA molecule by addition of a separate 5S-containing RNP (51). Eventually, the 40S and 60S subunits exit the nucleus into the cytoplasm where they assemble with additional ribosomal proteins. While much is known about mechanisms that regulate synthesis of individual ribosomal components (4, 56), we envision equally important mechanisms that promote efficient assembly of ribosomal components. Therefore, we wish to determine the driving forces that bring about exchange of ribosomal components between the nucleus and cytoplasm.

Mechanisms of macromolecular transport across the nuclear-cytoplasmic boundary have been the focus of much recent research. A conspicuous structure spanning the nuclear envelope, the nuclear pore complex (NPC),[1] was known to contain an aqueous channel ~90 Å in diameter through which small molecules could diffuse (44). However, many proteins that accumulate within the nucleus are much larger than the NPC channel diameter available to free diffusion, suggesting that facilitated uptake through nuclear pores must occur. Likewise ribonucleoprotein complexes such as ribosomal subunits or complexes containing mRNA are much larger than the passive diffusion limit of the NPC channel, so that exit of RNP's from the nucleus must be facilitated (see reference 29 for a recent review).

Facilitated exchange across the nuclear envelope implies that transported macromolecules possess particular properties that are recognized to promote transport. Feldherr and co-workers showed that inert gold particles coated with the Xenopus nuclear protein nucleoplasmin rapidly entered the nucleus after injection into the cytoplasm, demonstrating that nucleoplasmin contains specific information directing its transport (25). The gold particles were observed in the electron microscope to enter the nucleus by way of nuclear

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1. *Abbreviations used in this paper:* GlcNAc, N-acetyl glucosamine; NPC, nuclear pore complex; WGA, wheat germ agglutinin.

pores, thus identifying definitively the site of nuclear protein import. Furthermore, particles >200 Å in diameter entered through central channels in the pore complex, indicating that the NPC does indeed transport molecules much larger than its passive diffusion limit (25). Recent experiments using cryoelectron microscopy and image analysis suggest that an NPC transporter undergoes a structural rearrangement during transport presumably to accommodate large entities (3). Dingwall et al. were first to show that a discrete COOH-terminal domain of nucleoplasmin was necessary and sufficient to bring about its facilitated import into the nucleus (18). Presently many additional so called nuclear localization sequences or domains have been defined in other nuclear proteins (see introduction to reference 55 for a recent listing). These nuclear localization sequences, which are generally basic in amino acid composition and may be as few as five to seven residues, appear to be ligands for nuclear and cytosolic receptors that comprise a multistep transport process (1, 11, 40, 49, 58); subsequent to receptor binding, proteins containing nuclear localization sequences are translocated into the nucleus in a separate, energy-dependent step (43, 46).

The specific details of RNP export from the nucleus are less well defined than those of protein uptake. RNP export is probably associated with the NPC, a process exemplified most vividly in electron micrographs showing migration of the 400 Å Balbiani ring RNP granules through the central channel of pore complexes in *Chironomus* salivary gland cells (42, 52). EM studies also suggest that the same RNP adopt an extended, less compact conformation during passage through the channel (42). Thus RNP transport must provide for specific recognition of RNP complexes, either by the NPC itself or by other components that deliver RNPs to the pore complex, and transport may include active mechanisms that deform the particles.

By using isolated nuclei many groups have shown that release of endogenous RNP complexes is temperature and energy dependent, suggesting active transport (2, 15). The in vitro rate of RNP efflux has been correlated with the level of activity of a particular nuclear envelope NTPase; the NTPase is stimulated by exogenous RNA but its exact function is unknown (2, 7, 15). Further evidence for a facilitated transport mechanism was obtained by Zasloff who showed by microinjection of tRNAs into *Xenopus* oocyte nuclei that the export rate was saturable and sensitive to temperature (60). Interestingly, particular tRNA molecules having certain nucleotide substitutions were exported less efficiently, suggesting a specific recognition process at a step before or during the actual translocation of the tRNA molecule to the cytoplasm (54). Dworetzky and Feldherr examined the translocation step itself by showing that colloidal gold coated with RNA rapidly emerged into the cytoplasm when injected into *Xenopus* oocyte nuclei (20). Export of RNA coated gold particles, which occurred through the central channel of the NPC, also displayed properties of a mediated process, since the transport rate was saturable and particles nearly three times the size of the NPC free diffusion limit were transported efficiently. Indeed, the RNA–gold complexes caused the central channel of the pores to expand to as much as 230 Å. Significantly, transport was independent of the type of RNA used to coat the gold colloids. Since RNP complexes vary in RNA sequence and protein composition, this result

may suggest that facilitated nuclear export of RNP's could depend on a collective property, perhaps the presence of RNA exposed on the surface. A role for surface exposed RNA in RNP transport was also predicted by Clawson and Smuckler in a theoretical treatment of in vitro nuclear RNA release data (14).

To analyze the mechanism of ribosome RNP export from the nucleus we have analyzed an in vivo transport assay using microinjection of ribosomal subunit into *Xenopus laevis* oocyte nuclei. This procedure is free of many drawbacks such as swelling, extensive loss of protein, and contamination with cytoplasmic RNP that accompany the use of isolated nuclei (2, 15). Furthermore, since transport is initiated by microinjection, the assay is not limited to quantitation of release of preformed endogenous complexes without control over their composition or interaction with possible transport facilitating components.

Herein we describe experiments which demonstrate that ribosomal subunits injected into *Xenopus* oocyte nuclei exit the nucleus as intact particles and at physiologically normal rates. We also present evidence that export occurred by an active process mediated by the NPC. Thus, sucrose gradient sedimentation and denaturing gel electrophoresis showed that the transported material remained as ribosomal subunits. The rate of transport exhibited saturable kinetics and transport was inhibited by lowering the temperature or depleting cells of ATP. Transport of injected subunits was also blocked by prior or simultaneous injection of wheat germ agglutinin (WGA), a lectin previously shown to inhibit active nuclear transport by binding to glycoproteins present in the NPC. Finally, we found that prokaryotic ribosomal subunits and subunits from yeast were efficiently transported from *Xenopus* nuclei; transport of heterologous subunits also displayed saturation kinetics and inhibition either by low temperature, ATP depletion, or WGA. These latter results support the idea that a general biochemical feature of RNP's may direct their translocation to the cytoplasm.

Materials and Methods

Frogs, Cells, and Reagents

Female *Xenopus laevis* frogs were purchased from Xenopus I (Ann Arbor, MI) and maintained at 19°C in distilled water that was replaced every 2 d; frogs were fed raw calf liver. A *Xenopus laevis* kidney tissue culture cell line, the generous gift of Dr. Igor Dawid (National Institutes of Health, Bethesda, MD), was grown in Eagle's MEM medium at room temperature under ambient atmosphere. Apyrase (grade VIII) and wheat germ agglutinin were from Sigma Chemical Co. (St. Louis, MO). An ATP bioluminescence assay kit was obtained from Boehringer Mannheim Diagnostics Inc. (Houston, TX).

Synthesis and Isolation of Radiolabeled Ribosomal Subunits

Xenopus laevis kidney cells were labeled in a 75-cm^2 flask during a 2-d period by transferring cells to 10 ml of Eagle's MEM medium prepared without methionine and phosphate and containing 1 mCi of $^{32}PO_4$ and/or 1 mCi of L-[^{35}S]methionine. After several rinses with cold PBS, the cells were scraped from the flask and washed once in PBS. Cells were lysed by incubation for 15 min at 4°C in ribosome isolation buffer (0.25 M sucrose, 35 mM KCl, 1.5 mM MgCl$_2$, 50 mM Tris-HCl, pH 7.6, 0.5% NP-40). The lysate was centrifuged 15 min at 35,000 g; ribosomes were recovered from the supernatant by centrifugation for 3 h at 120,000 g. Ribosomal subunits were prepared by gently resuspending the pellet in 50 mM Tris-HCl, pH 7.6, 500 mM KCl, 1 mM MgCl$_2$, followed by centrifugation through a

10-40% sucrose gradient prepared in the same buffer. Fractions were collected with a density gradient fractionator (model 640; ISCO, Inc., Lincoln, NE) equipped with a UV monitor; fractions containing ribosomal subunits were pooled and the subunits were pelleted at 4°C, for 20 h at 180,000 g before microinjection. Pellets were resuspended in intracellular medium (102 mM KCl, 11.1 mM NaCl, 7.2 mM K_2HPO_4, 4.8 mM KH_2PO_4, pH 7.0; reference 20). Ribosomes from injected oocytes were analyzed by disrupting the cells in 5 ml ribosome isolation buffer using a Teflon-coated pestle in a tissue homogenizer. Extraction of ribosomes and sucrose gradient centrifugation were as described for cell culture extracts.

Escherichia coli and Saccharomyces cerevisiae cells were grown to logarithmic phase and labeled for 2 h with 2 mCi of $^{32}PO_4$ in 50 ml of standard complex media that had been depleted of inorganic phosphate (47). E. coli ribosomes were prepared by incubating cells for 10 min with lysozyme (1 mg/ml) and Na_2EDTA (60 mM) in 10% sucrose-50 mM Tris-HCl, pH 8. After incubation, cells were pelleted and lysed by resuspending in TMKD buffer (20 mM Tris-HCl, pH 8, 10 mM $MgCl_2$, 50 mM K acetate, 10 mM DTT) containing 10 mg/ml saponin and 5 μg/ml DNAse I, followed by two cycles of freezing and thawing. Lysed cells were diluted by a factor of three and centrifuged 15,000 g 10 min. The supernatant was layered on a cushion of 10% sucrose in lysis buffer containing 5% $(NH_4)_2SO_4$ and centrifuged at 4°C for 4 h at 180,000 g. The pellet was resuspended in 25 mM Tris-HCl, pH 7.8, 500 mM KCl, 5 mM Mg acetate to dissociate subunits. The dissolved pellet (35-50 A_{260} units) was centrifuged at 67,000 g for 20 h through a 10-30% sucrose gradient in the same buffer. Subunits were recovered, pelleted, and resuspended as described for Xenopus subunits above. Yeast ribosomes were prepared from cells mechanically disrupted with glass beads in TKMD containing 200 mg/ml heparin. Ribosomes and ribosomal subunits were isolated from yeast extracts essentially as described above for E. coli.

RNP specific activity (expressed in cpm/μg of RNA) was determined by measuring the A_{260} and cpm of phenol-extracted RNA obtained from an aliquot of each ribosomal subunit preparation.

RNA Purification and Analysis

RNA was prepared as described by Probst et al. (45). Oocytes were homogenized quickly in homogenization buffer (2% [wt/vol] sodium dodecyl sulfate, 0.3 M NaCl, 50 mM Tris pH 8.0, 1 mM Na_2EDTA) and then extracted with phenol/chloroform. RNA was precipitated with ethanol and analyzed by electrophoresis in 1.2% (wt/vol) agarose gels containing 1.1 M formaldehyde (41).

Intranuclear Injections and Oocyte Dissection

Frogs were anesthetized on ice for 45 min and the ovaries surgically removed. Stages V and VI oocytes were manually defolliculated with watchmakers forceps and maintained in MBS medium (32) at 19°C. Before injection, oocytes were centrifuged at 650 g for 8-10 min (20, 38). During centrifugation the germinal vesicle migrated to the surface of the animal hemisphere and could be easily visualized as a distinct targetlike structure. The nuclei were microinjected within 15 min after centrifugation, using calibrated needles (10 μm o.d.) delivering 20 nl. The injected oocytes were maintained in MBS medium at 19°C until further analysis.

Despite the targetlike appearance of the germinal vesicle, it was impossible to determine with 100% confidence that the tip of the needle was strictly within the nucleus during injection. Thus, the ribosomal subunit samples were coinjected with colloidal gold particles coated with polyvinylpyrrolidone (PVP) to monitor the actual site of injection. The colloidal gold suspension displayed a red color so that after injection and dissection of the oocytes to separate nuclei from cytoplasm, only nuclei that were stained red were analyzed. Colloidal gold particles were prepared by reducing chloroauric acid with a saturated solution of white phosphorus in ether (23), yielding particles of 20-50 Å diameter. These particles were stabilized with polyvinylpyrrolidone, concentrated in an Amicon concentrator, and dialyzed against intracellular medium (20). Dworetzky and Feldherr demonstrated that after nuclear injection, PVP-coated gold particles were retained within the nucleus and less than 6% of the pores contained gold particles (20).

Manual dissection of oocytes was performed as described (24). After dissection, nuclei were removed in <30 s into intracellular medium. The nuclei and cytoplasms were fixed in ethanol if they were to be used for counting radioactivity or they were quick frozen in homogenization media if used for RNA purification.

Radioactivity Measurements

Radioactivity distribution in the oocytes was determined as follows: individually ethanol fixed nuclei and cytoplasm were dried and rehydrated in 10 μl of distilled water, then hydrolyzed 1 h at 60°C in 300 μl of Nuclear Chicago Solubilizer (Amersham Corp., Arlington Heights, IL). The radioactivity content of each sample was determined after addition of 5 ml of Cytoscint ES (ICN Biomedicals, Costa Mesa, CA) by counting in a scintillation counter.

ATP Measurements

Either whole oocytes or separated nuclei and cytoplasms were individually mixed with 0.5 ml boiling 20 mM Hepes buffer (pH 7.5) and kept at 100°C for 5 min. Thereafter, samples were kept at 4°C and analyzed the same day. ATP was measured with the ATP bioluminescence CLS kit (Boehringer Mannheim Diagnostics Inc.). Samples were diluted 500-fold in H_2O and 500 μl of the diluted sample was added to the luciferase-luciferin reagent solution before counting in a scintillation counter. Five consecutive 12-s counts were taken and averaged.

Results

Microinjected Ribosomal Subunits Are Transported from Nuclei as Intact Particles

The purpose of this study was to characterize an in vivo assay for nuclear export of ribosomal ribonucleoprotein complexes, an assay that should be representative of the normal physiological process and amenable to biochemical intervention. We chose as an assay injection of radiolabeled Xenopus laevis ribosomal subunits into Xenopus oocyte nuclei, observing export as the appearance of radioactivity in the cytoplasm. For this procedure it was essential to determine that radioactivity appearing in the cytoplasm was in the form of intact ribosomal subunits. This verification was achieved in two ways.

First, we injected ^{32}P-labeled subunits as well as 80S ribosomes into the nuclei of 20-30 oocytes. After 2 h of incubation at 19°C the oocytes were manually dissected and RNA was extracted from the pooled nuclei and cytoplasmic fractions. The RNA samples were subjected to gel electrophoresis under denaturing conditions. As a control for the possibility that degradation might occur after translocation through the nuclear envelope, ribosomal subunits were injected directly into the cytoplasm and the oocytes incubated for 2 h before extraction of total RNA. Autoradiograms of the gels are presented in Fig. 1. As is clearly evident, after nuclear injection of labeled ribosomal subunits, ^{32}P-labeled RNA extracted from either the nucleus or the cytoplasm displayed the same electrophoretic mobility as RNA extracted from the ribosomal samples before microinjection. No significant differences were seen for injected 40S or 60S subunits or 80S ribosomes. The RNA obtained from ribosomal samples injected directly into the cytoplasm was also recovered intact; note that no radioactivity was detected in nuclei from cells in which ribosomal subunits had been injected directly into the cytoplasm (not shown). These results indicated that at least the RNA moieties of nuclear injected Xenopus ribosomal subunits emerged intact into the cytoplasm. Since only a single species of labeled rRNA was recovered when either 40S or 60S subunits were injected (Fig. 1 B), the labeled rRNA in the cytoplasm was not due to hydrolysis of the injected material followed by resynthesis of rRNA, in which case both radiolabeled 18S and 28S rRNA molecules would have been observed.
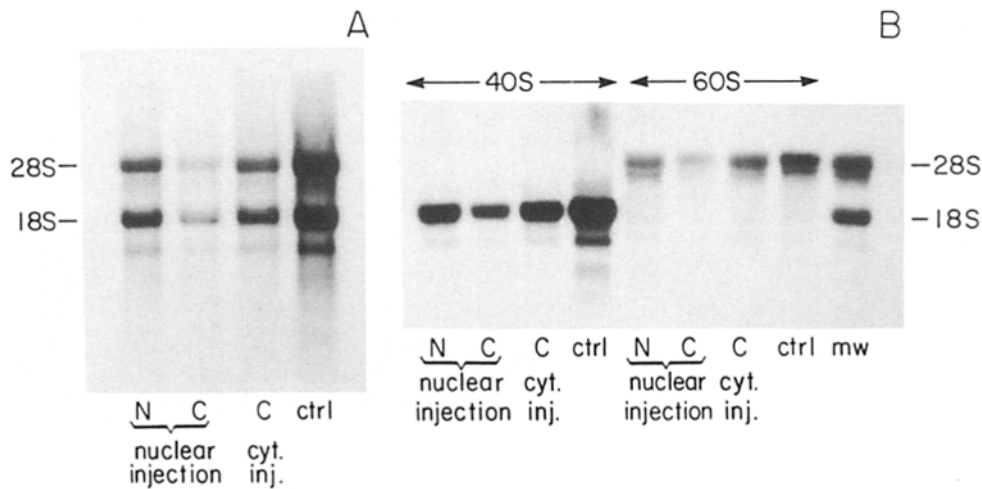
Figure 1. Gel electrophoresis of ^{32}P-labeled RNA recovered from *Xenopus* oocytes after microinjection of radiolabeled *Xenopus* ribosomes or ribosomal subunits. Separated 40S and 60S ribosomal subunits or 80S monosomes were injected either into the nucleus or cytoplasm of *Xenopus* oocytes. Subunits representing ∼10 ng (2.5–3 × 10² cpm/ng) of rRNA (5 ng was a saturating amount; see Fig. 3) were injected per oocyte. Injected oocytes were incubated at 19°C and 2 h postinjection were manually dissected, to separate nuclei from cytoplasms, and quick frozen; RNA was extracted from pooled nuclear and cytoplasmic fractions as described in Materials and Methods. The RNA was denatured with formaldehyde and separated by size in 1.2% agarose gels. Gels were dried and subjected to autoradiography. Each lane on the gel contained the total RNA recovered from about three nuclei or cytoplasms of injected cells, respectively. (Several uninjected oocytes were mixed with the nuclei before extraction to provide sufficient unlabeled RNA as carrier and for visualization of the RNA by UV in the gels.) *N*, RNA extracted from nuclei; *C*, RNA extracted from cytoplasm; *nuclear injection* indicates that ribosomal material was injected initially into nuclei; *cyt. inj.*, cytoplasmic injection; *ctrl*, RNA extracted from uninjected ribosomal sample. *A* shows results for injection of 80S ribosomes; *B* shows results for injection of either 40S or 60S subunits.

A more critical test of integrity of the transported material was to determine if the injected subunits were recoverable as complexes of ribosomal proteins and rRNA. Thus, we injected into oocyte nuclei ribosomal subunits that had been dual labeled with ^{32}P and ^{35}S; after 2 h of incubation at 19°C, ribosomes were pelleted from pooled oocyte cytoplasms and sedimented (under conditions that prevent subunit association) in sucrose gradients. The ^{32}P and ^{35}S content across the gradients was determined by TCA precipitation and compared to the sedimentation profile (A_{260}) of the endogenous (unlabeled) ribosomal material. Fig. 2 shows that the peaks of ^{32}P and ^{35}S in the gradients were superimposable upon each other, demonstrating that material recovered from the cytoplasm after nuclear injection was in the form of ribonucleoprotein complexes. Also, the ^{32}P/^{35}S ratios for the peak fractions in the gradients were the same as the ^{32}P/^{35}S ratios of the original uninjected ribosomal subunits; this result proved that the RNP complexes in the cytoplasm could not have resulted from, for example, disassembly of the injected subunits followed by reassembly of the radiolabeled components with endogenous (unlabeled) components to create new transport-competent RNPs. Finally, the peaks of radioactivity in the gradients corresponded approximately to 40S (Fig. 2 *A*) and 60S (Fig. 2 *B*) demonstrating that the transported ribosomal RNPs had retained essentially the same overall structures as the original uninjected subunits. Actually, the ^{32}P/^{35}S peaks occurred at a slightly more dense position in the gradient relative to 40S and 60S subunits; this density variation between injected (cytoplasmic recovered) and uninjected subunits was reproducible but we do not know if the difference was artifactual or the result of a meaningful physiological modification (see Discussion). In conclusion this ensemble of results demonstrated that ribosomes injected into the oocytes were not degraded nor grossly altered and that the measure of radioactivity in the two cellular compartments after injection of radiolabeled ribosomes was a reliable indication of the intracellular distribution of the RNP particles.

Ribosome Export Is a Saturable Process

Having established that ribosomal subunits injected into *Xenopus* oocyte nuclei emerged intact into the cytoplasm, we wished next to determine whether this transport occurred by a deliberate facilitated process. Kinetically, a mediated transport process is expected to be one whose rate is proportional to the concentration of the transported material, reaching a maximum when all carriers are saturated; in contrast, transport by simple diffusion is generally unsaturable (13). Thus, to determine the nature of export in our assay, we injected into oocyte nuclei increasing amounts of ^{32}P-labeled 60S *Xenopus* ribosomal subunits and determined the time required for export of 50% of the injected material. As shown in Fig. 3, the rate of transport increased with increasing amounts of injected subunits, reaching a maximal rate of 0.04 ng RNA/min per oocyte or ∼1.7 × 10⁷ 60S subunits/min per oocyte. Export of 40S subunits displayed virtually identical kinetics (data not shown). These results suggested that export of injected subunits was dependent upon a limiting component of a facilitated transport process. In an independent experiment, ^{3}H-labeled mouse IgG was microinjected into nuclei; the complete absence of radioactivity in the cytoplasm after several hours of incubation provided a control that the technique of microinjection did not induce major leakage of intranuclear material (data not shown).

Export of Heterologous Ribosomal Subunits

In eukaryotic cells many types of RNP complexes differing in size, protein composition and RNA species are transported out of the nucleus into the cytoplasm. Given their complexity, perhaps some general biochemical feature shared by RNP complexes might promote their export. This possi-
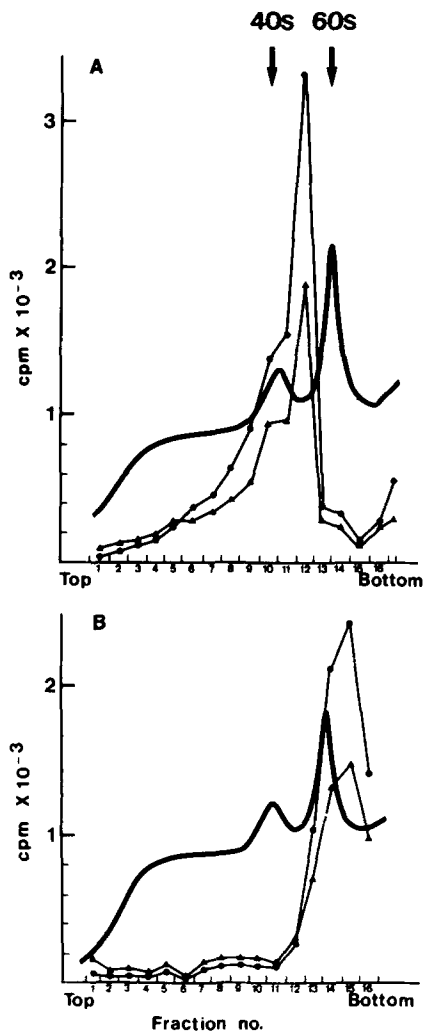
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40S 60S

Figure 2. Sucrose density gradient centrifugation of oocyte extracts after nuclear injection of *Xenopus* ribosomal subunits. Individual 40S and 60S *Xenopus* ribosomal subunits, labeled with both ^{32}P and ^{35}S (2.5–3 × 10^2 cpm/ng rRNA), were injected into oocyte nuclei (10 ng rRNA injected per oocyte; 50 oocytes injected for each subunit). 2-h postinjection oocytes were manually dissected and pooled cytoplasms were extracted and sedimented in sucrose gradients as described in Materials and Methods. Gradient fractions (0.6 ml) were collected and assayed for radioactivity by diluting with 5 ml 10% TCA, collecting the resulting precipitates by filtration onto GF/C filters (Whatman Inc., Clifton, NJ), and counting the filters in a scintillation counter. The solid curve in each graph is the A$_{260}$ profile of the endogenous unlabeled ribosomal subunits. *A* presents results for injected 40S ribosomal subunits and *B* for 60S subunits. (●) ^{32}P; (▲) ^{35}S.

bility is supported by the observation that colloidal gold coated with any type of RNA was efficiently exported from *Xenopus* oocyte nuclei (20). Thus, we wished to determine if ribosomal subunits from sources other than *Xenopus* could be exported from *Xenopus* oocyte nuclei. As evidenced from RNA gel analyses, Fig. 4 shows that indeed ribosomal RNPs from either yeast or bacteria were exported to the cytoplasm after *Xenopus* nuclear injection. The ^{32}P rRNA species recovered from the cytoplasms had gel mobilities corresponding to those of the original yeast or *E. coli* species (all of which migrated faster than their *Xenopus* counterparts), in-

dicating that export of heterologous subunits could not have been due to breakdown of the foreign subunits and resynthesis into *Xenopus* rRNA.

As further evidence for transport of intact heterologous subunits, we subjected cytoplasmic extracts to sucrose gradient sedimentation after injecting either dual-labeled *E. coli* 30S and 50S subunits into nuclei. As is seen in Fig. 5, the peaks of radioactivity in the gradients differed from (were less dense than) the 40S and 60S peaks of the endogenous *Xenopus* ribosomal subunits and corresponded exactly to the sedimentation of the uninjected 30S and 50S *E. coli* subunits (not shown). In addition, the ^{32}P and ^{35}S cosedimented in the gradients; these results demonstrated that the *E. coli* rRNAs and their associated ribosomal proteins were transported as partners and intact.

Still further evidence for export of heterologous RNPs was obtained from measurement of the transport rate of *E. coli* 30S subunits as a function of the number of injected subunits. Fig. 3 shows that export of prokaryotic subunits in *Xenopus* exhibited saturable kinetics comparable to that for eukaryotic ribosomes, again indicating facilitated transport. Actually, the maximum export rate of *E. coli* 30S subunits was nearly 18 times greater than that of *Xenopus* 60S subunits (0.25 ng/min = 3 × 10^8 subunits/min; compare with 1.7 × 10^7 subunits/min for *Xenopus*). Perhaps a slightly greater export rate might be expected for the *E. coli* 30S RNP due to it being less than half the size of the eukaryotic 60S particle, but without more mechanistic information we can not account for this large rate difference. Nonetheless as described below, transport of *E. coli* subunits was equally affected by conditions that blocked transport of *Xenopus* subunits, so export of heterologous ribosomal subunits in *Xenopus* oocytes presumably operated by the same pathway as for *Xenopus* ribosomal subunits.
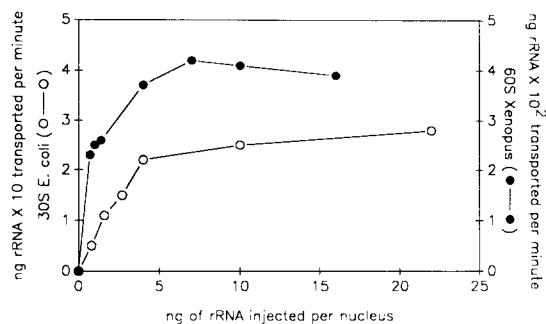

Figure 3. Ribosome export is a saturable process. The rate of transport as a function of the amount of injected subunits was determined as follows: Oocytes were injected with different amounts of ^{32}P-labeled ribosomal subunits (expressed as ng rRNA) and at various times after injection the percent radioactivity in the cytoplasm was determined for 5–10 oocytes. The mean value for each set of 5–10 oocytes per time point was plotted and the $t_{1/2}$ of export (ng rRNA transported per minute) determined from the linear regression line through these points. The standard deviation was generally <10% of the mean value for each time point. The graph shows the $t_{1/2}$ values plotted as a function of amount injected. Note the difference in abscissa scale between *Xenopus* subunits and *E. coli* subunits. Ribosome specific activity: *Xenopus*, 2.5–3 × 10^2 cpm/ng; *E. coli*, 3.5–4.0 × 10^2 cpm/ng.
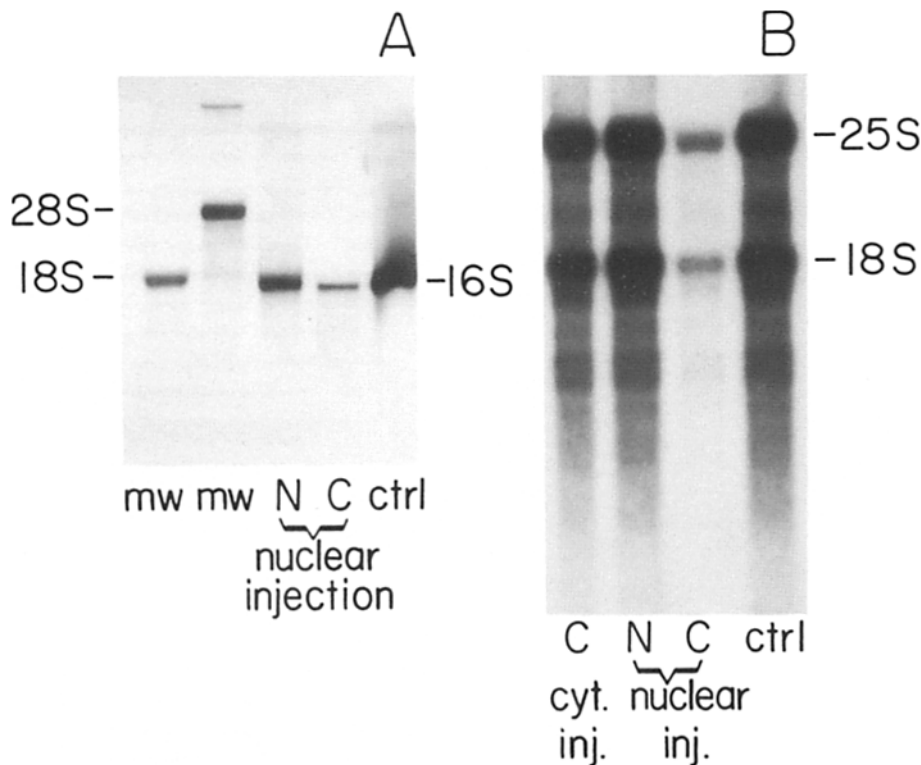
Figure 4. RNA analysis of oocytes injected with *E. coli* 30S subunits and *Saccharomyces cerevisiae* 80S ribosomes. (*A*) ^{32}P-labeled *E. coli* 30S ribosomal subunits (3.5–4.0 × 10^2 cpm/ng) were microinjected into the nuclei of 20–30 oocytes. After 2 h at 19°C oocytes were manually dissected. RNA was extracted and analyzed by denaturing gel electrophoresis as described in Materials and Methods and Fig. 1. *mw*, *Xenopus* rRNA molecular weight markers; other labels are as in Fig. 1. (*B*) ^{32}P-labeled 80S yeast ribosomes (2 × 10^2 cpm/ng) were injected and RNA analyzed as above.

Temperature Dependence of Ribosomal Subunit Export

The previous results showing saturation kinetics indicated that export of microinjected ribosomal subunits was a facilitated process. Temperature dependence has also been used to distinguish mediated from diffusional transport. Facilitated nuclear import of karyophilic proteins is reversibly inhibited by low temperature (11, 46). In vivo export of tRNA molecules from *Xenopus* oocyte nuclei and in vivo efflux of RNP complexes from isolated nuclei were shown also to be temperature dependent (15, 60). To determine the temperature dependence of ribosomal subunit export, ^{32}P-labeled *Xenopus* or *E. coli* ribosomal subunits were injected into *Xenopus* oocyte nuclei. After various periods of incubation, at either 19 or 4°C, the oocytes were manually dissected and the radioactivity in the nucleus and cytoplasm measured for each oocyte. Fig. 6 shows that at 19°C ribosomal subunits were released from the nucleus for 60–90 min after injection, at which time a plateau was reached. About 80% of the subunits appeared in the cytoplasm by 90 min. At 4°C the subunits remained in the nucleus and, even after 3 h of incubation, <20% of the radioactivity was recovered in the cytoplasmic fractions. Inhibition of export by low temperature was completely reversible upon warming of the cells (Fig. 7). Since the magnitude of the temperature effect observed exceeded the expected impact that the lower temperature would have on a simple nonspecific process such as diffusion (13), these results were consistent with the view that a facilitated mechanism was involved in export of the injected subunits.

Export of Ribosomal Subunits Is Inhibited by ATP Depletion

The temperature dependence for nuclear export suggested a facilitated (enzymological) process, an observation not un-

expected given that the RNP complexes are at least twice the size of the NPC channel available to free diffusion. Nuclear protein import has been shown to require ATP in vivo and in vitro (43, 46) and in vitro efflux of RNP from isolated nuclei has been correlated with ATP consumption and the activity of a particular nuclear envelope NTPase (2, 15). We therefore investigated the requirement for ATP in our in vivo export assay.

Xenopus oocytes, which normally contain ATP at 1–2 mM, were depleted of ATP by microinjection of the ATPase/ADPase enzyme apyrase (12, 43). Injection of apyrase at a final concentration of 20 U/ml (20 nl/oocyte) resulted in a rapid decline in ATP concentration as determined by a luciferase assay. Under these conditions, oocyte intracellular ATP concentration was reduced to background levels (<0.1 mM) within the 30 min after injection. However, we wish to note two important points regarding apyrase treatment. First, as a consequence of ATP depletion the oocyte cytoplasm became quite fluid, probably due to abolition of ATP-dependent actomyosin-gelation contraction (21). Thus, in the series of experiments involving apyrase described below, oocytes depleted of ATP were fixed in 1% TCA before dissection, to avoid major loss of cytoplasmic contents during enucleation. Secondly, we determined separately the ATP concentration in the nuclear and cytoplasmic compartments, after injection of apyrase either in the nucleus or in the cytoplasm. The pools of ATP in the two compartments were not in equilibrium. 1 hour after nuclear injection of apyrase, the ATP concentration in this compartment was reduced from 1–2 mM to <0.15 mM whereas ATP was still present at 2 mM in the cytoplasm; also, nuclear injection of apyrase also did not cause the cytoplasm to liquefy. Conversely, injection of apyrase into the cytoplasm, while depleting this compartment of ATP and inducing considerable fluidity, did not affect the ATP level in the nucleus.
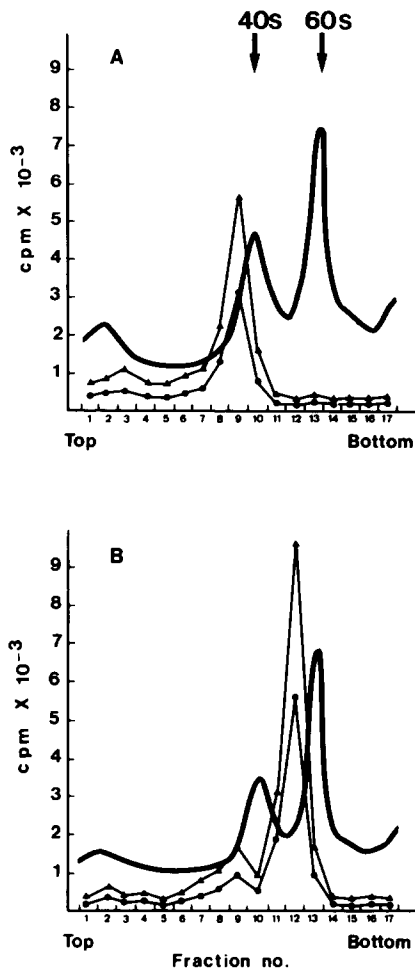
Figure 5. Sucrose density gradient centrifugation of oocyte extracts after nuclear injection of *E. coli* ribosomal subunits. Analysis was performed as described in Fig. 2. Amount of injected subunits = 10 ng rRNA/oocyte (2.5-3 × 10² cpm/ng RNA; 100% saturation). A total of 60 oocytes were injected for each subunit. *A* presents results for injected 30S ribosomal subunits and *B* for 50S subunits. (●) ³²P; (▲) ³⁵S.
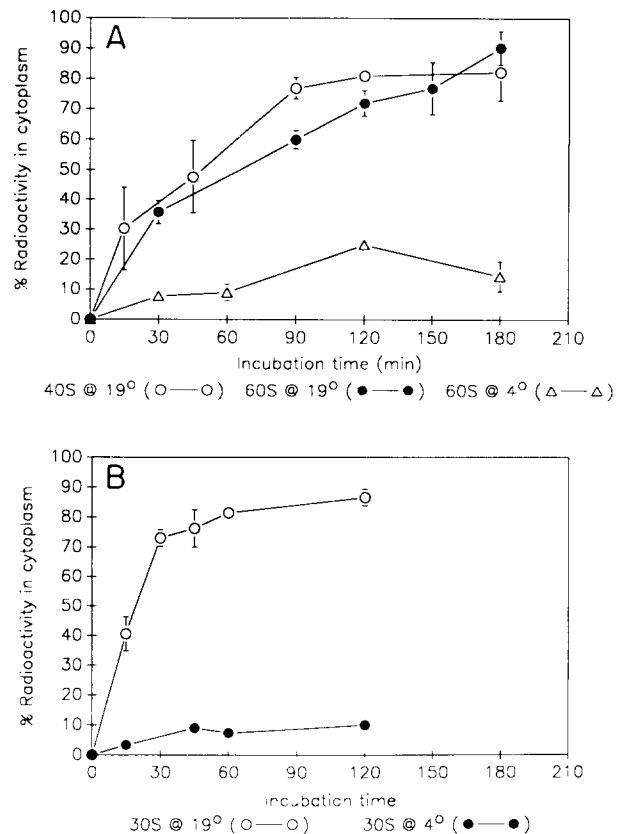

Figure 6. Temperature dependence of ribosomal export in *Xenopus* oocytes. Oocytes preincubated at 19 or 4°C were injected with ribosomal subunits from (*A*) *Xenopus* (4 ng/oocyte; 2.5 × 10² cpm/ng rRNA; 80% of saturation) or (*B*) *E. coli* (2.5 ng/oocyte; 3.75 × 10² cpm/ng rRNA; ∼33% saturation). The injected oocytes were maintained at their initial temperature (4°C oocytes were prevented from warming during injection by placing the petri dish that held the cells on a metal block immersed in ice water). The graphs show the percent of total injected radioactivity that was found in the cytoplasm after various time periods (radioactivity measurements were determined on individual cells; each time point consisted of an average of four to five oocytes).

Having established parameters for ATP depletion, radiolabeled ribosomal subunits were injected into oocyte nuclei along with apyrase, or immediately after apyrase was injected into the cytoplasm. The oocytes were incubated at 19°C for various time periods after injection and then manually dissected. As shown in Fig. 8, depletion of ATP in the nuclear compartment resulted in a dramatic inhibition of ribosome export; this inhibition occurred within a few minutes after injection. Less than 10% of the radioactivity was recovered in the cytoplasm, even after 1 h of incubation. Identical results were found for *E. coli* ribosomal subunits (not shown). On the other hand elimination of ATP in the cytoplasm had no effect on the exit of nuclear injected ribosomes (not shown). While the manner in which ATP was used is unknown, these results further supported the conclusion that export of injected ribosomal RNPs is a facilitated process.

Export of Injected Ribosomes Is a Nuclear Pore–associated Process

Recently it has become clear that the NPC of higher eukary-

otes contains a group of proteins having an unusual form of O-linked glycosylation (17, 27, 34, 35, 50). Moreover, either WGA or a monoclonal antibody specific for these glycosylated proteins has been shown to inhibit nuclear protein import in vivo and in vitro (16, 22, 27, 57, 59). Inhibition of transport was not the result of a simple steric blockade by WGA or antibody bound to the NPC because smaller molecules were still able to diffuse into the nucleus; rather it has been postulated that lectin or antibody binding to these glycosylated NPC proteins prevents the pore proteins from carrying out their normal role in active transport. The same conclusion derives from recent in vitro import experiments in which NPCs assembled successfully from extracts depleted of WGA-binding proteins but the resulting NPCs were incapable of active transport (26).

Since inhibition of transport by WGA is suggestive of an active NPC-mediated process, we sought to characterize further the export of microinjected ribosomal subunits by measuring the rate of export of *Xenopus* subunits in the presence of WGA. WGA was injected at a final concentration of 0.5 mg/ml either into the nucleus or into the cytoplasm of *Xeno-*
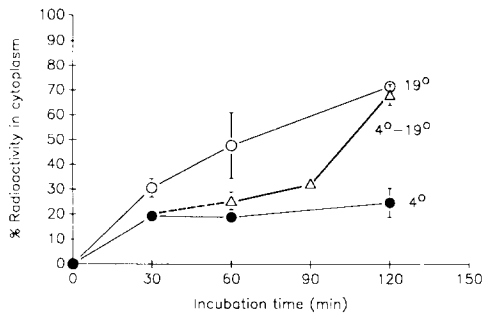
Figure 7. Reversibility of low temperature inhibition of ribosome export. As described in the legend to Fig. 6, *E. coli* 30S ribosomal subunits (1.5 ng/oocyte; 2.5 × 10² cpm/ng rRNA; ~15–30% saturation) were injected into nuclei of oocytes preincubated at either 19 or 4°C. After 30 min, one-half of the oocytes injected at 4°C were shifted to 19°C.

pus oocytes. When WGA was injected into the cytoplasm and oocytes incubated for either 1 or 3 h before subsequent nuclear injection of radiolabeled ribosomal subunits, the kinetics of export was unaltered compared to control oocytes preinjected with buffer (data not shown). The results for injection of WGA into the nuclei were quite different (Fig. 9 *A*). WGA was either coinjected with the ribosomal subunits into the nucleus or injected into the nucleus 1 h before injection of ribosomal subunits. When WGA and subunits were coinjected, inhibition of ribosomal export was evident after 1 h and by 3 h 40% of the radioactivity remained in the nuclear compartment. Presumably, the delay in inhibition was due to the time required for the WGA to move through the nucleoplasm and bind to the NPCs. When WGA was injected in the nucleus 1 h before injecting ribosomal subunits, inhibition of export was more immediate although still incomplete; in this case, ~60% of the radioactivity remained in the nucleus 3 h after injection. The fact that inhibition of export was more rapid when WGA was injected 1 h before the
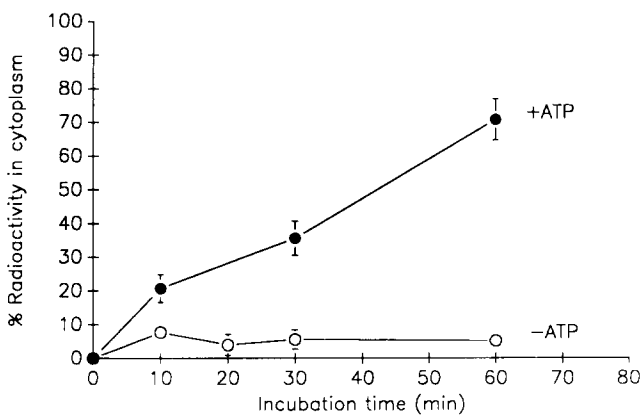

Figure 8. ATP dependence of ribosome export in *Xenopus* oocytes. Oocyte nuclei were depleted of ATP with apyrase as described in Materials and Methods. ³²P-labeled 60S ribosomal subunits (2.5 ng/oocyte; 1.8 × 10² cpm/ng; ~40% saturation) were mixed with apyrase or buffer and injected into nuclei. After various time periods, cells were fixed in TCA (see Results) and dissected manually; the percent of total radioactivity found in the cytoplasm of individual cells was determined.

ribosomal subunits implies that inhibition was due to the interaction of WGA with nuclear components and not due to an interaction between WGA and the ribosomal subunits. These results therefore suggested that export of microinjected ribosomal subunits occurred via the nuclear pore complex.

As shown above, ribosomes from other sources were exported from oocyte nuclei. To verify that heterologous export was also likely an NPC-associated process, we assayed transport of *E. coli* 30S ribosomal subunits in nuclei preinjected with WGA (0.75 mg/ml). Like *Xenopus* subunits, microinjected *E. coli* subunits were prevented from export to the cytoplasm by WGA (Fig. 9 *B*). In this experiment we actually observed greater inhibition than was measured in the case of injected *Xenopus* subunits, although this effect may have been due to the 50% higher concentration of WGA used in the latter experiment. Finally, WGA is specific for *N*-acetyl glucosamine residues (GlcNAc). To verify that inhibition of export resulted from WGA interacting with GlcNAc-containing glycoproteins, we coinjected WGA with GlcNAc; Fig. 9 *B* shows that the presence of 50 mM competing GlcNAc abolished WGA induced inhibition of export.

Discussion

The purpose of this study was to characterize in depth the use of radiolabeled ribosomal subunits microinjected into nuclei as an assay to investigate the biochemical properties of RNP transport. The results presented here illustrate that ribosomal subunits injected into *Xenopus laevis* oocyte nuclei were exported in a physiologically representative fashion. First, as much as 90% of the injected radioactivity appeared in the cytoplasm 2–3 h postinjection. Denaturing gel electrophoresis and sucrose gradient centrifugation demonstrated that radioactivity in the cytoplasmic fraction was in the form of intact ribonucleoprotein complexes that had essentially the same sedimentation properties as the original ribosomal subunits. Ribosomal material could not be detected entering the nucleus after cytoplasmic injection, which is consistent with the normal unidirectional transport of endogenous ribosomes. Secondly, the rate of export of injected subunits was concentration dependent and saturable, properties indicative of a mediated process rather than one that occurred by simple diffusion. Given that endogenous RNP complexes are two to three times the size available to free diffusion through the NPC channel, some form of facilitated RNP transport is expected (2, 15). We further substantiated that export of injected subunits was a facilitated, energy-dependent process by showing that export was inhibited completely either by lowering the temperature or by depleting the nuclei of ATP; the effect of lowered temperature was completely reversed upon warming the cells. Finally, we showed that export of injected ribosomal subunits was likely an NPC-associated process because export was inhibited in the presence of WGA, a lectin known to inactivate facilitated nuclear pore traffic by binding to O-glycosylated NPC proteins (16, 27, 49, 57). WGA induced inhibition of injected subunit transport was consistent with previous electron microscopic observations showing that export of either endogenous RNP or RNA-coated colloidal gold tracers takes place through the central channel of the NPC (20, 42, 52).

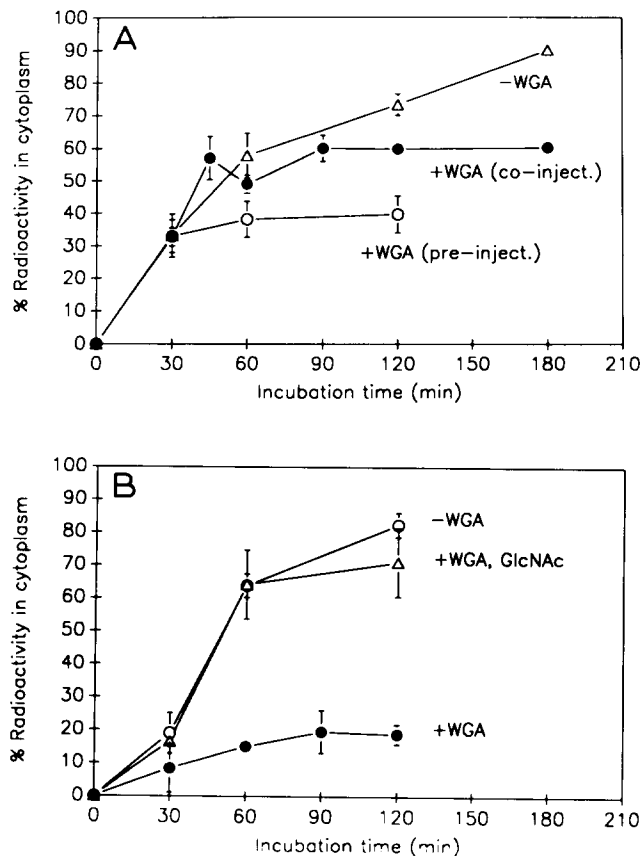In the experiments reported here, the export rate of in-

Figure 9. Effect of WGA on ribosome export. (*A*) Radiolabeled 60S *Xenopus* ribosomal subunits (2 ng/oocyte; 10^3 cpm/ng; ~40% saturation) were injected into oocyte nuclei that had been injected 1 h previously with WGA or the subunits were coinjected with WGA. The amount of WGA used was estimated to yield a final intranuclear concentration of 0.5 mg/ml. (*B*) *E. coli* 30S ribosomal subunits (5 ng/oocyte; 3.75 × 10^2 cpm/ng; ~70% saturation) were injected into oocyte nuclei previously injected either with WGA (final concentration 0.75 mg/ml) or with a mixture of WGA + GlcNAc (50 mM final concentration).

jected 40S or 60S *Xenopus* ribosomal subunits reached a maximum of ~1.7 × 10^7 subunits/min per oocyte (0.04 ng rRNA/min). A recent study similar to our own reported a maximal export rate for injected subunits ~25% of that observed in this investigation (30). Although no direct measurements have been made of the endogenous subunit export rate in *Xenopus* oocytes, Anderson and Smith (5) reported that mature oocytes accumulate about 0.65 ng ribosomal RNA per hour (0.01 ng/min). Assuming that endogenous 40S and 60S subunits are both transported at the same rate (which is probably not the case) and given that 70% of total rRNA is in the 60S subunit, the data reported by Anderson and Smith (5) would correspond to ~3 × 10^6 subunits exported per minute, or about one-sixth the maximum rate measured in our injection assay. Thus, while the export rate of injected subunits and the export rate predicted for endogenous subunits were not exactly equivalent, our results are within an expected, physiologically meaningful range, especially given the approximate nature of the calculations. Furthermore, the transport rate of endogenous ribosomal subunits is coupled to the rates of their synthesis and assembly,

either or both processes which could take place at less than the maximum rate sustainable by the transport apparatus. The injected subunits in our assay were, of course, not bound by constraints of prior synthesis and assembly.

The injected ribosomal subunits were shown by sucrose gradient centrifugation to have entered the cytoplasm as intact RNP complexes. Although the 30S and 50S *E. coli* subunits recovered from cytoplasms after nuclear injection sedimented identically compared to the same material before injection, both 40S and 60S *Xenopus* subunits recovered after injection sedimented just slightly faster than their endogenous counterparts (see Fig. 2), indicating some alteration in structure. This difference could simply have been an artifact of having twice isolated and sedimented the injected ribosomes. Alternatively, structural alterations may have occurred within the oocyte, in which case the modifications may still have been artifactual or indicative of a true physiological process. Ribosomal subunits are believed to mature through a process of protein exchange associated with their appearance in the cytoplasm (33, 36; see below). Similarly, mRNA molecules contained in heterogeneous nuclear RNP complexes shed their HnRNP proteins and acquire new proteins commensurate with transport to the cytoplasm (19). Thus it is possible that some reassortment of protein molecules may have occurred during export of injected ribosomal subunits, creating a slight change in sedimentation rate. The ratio of ^{32}P to ^{35}S for the labeled subunits rose slightly from 1.3 before injection to 1.6 after recovery from the cytoplasm. Further, there was consistently more ^{35}S than ^{32}P at the top of the gradients even though the input of ^{35}S was initially lower than the ^{35}P input. Both of these phenomena were consistent with the injected subunits undergoing some loss of protein during or after transport.

Metabolic energy, probably in the form of ATP, is required for the facilitated import of proteins into the nucleus (43, 46) and we have shown here a requirement for ATP in ribosomal RNP export. From the moment the NPC was identified and its biophysical properties determined, it was assumed that RNP export is an energy-dependent step. The source for this energy is widely believed to be a particular nuclear envelope NTPase which has been extensively purified and characterized (2, 48). At least for mRNA export, support for an involvement of this NTPase is based on numerous correlations showing that mRNP efflux and NTPase activity both exhibit the same kinetics, substrate specificity, and reaction condition requirements, they both show the same alterations during development and hormone treatment, they both display the same sensitivity to inhibitors, and they both are equally affected by stimulatory agents such as poly(A) or poly(A)$^+$ mRNA (see reference 48). Whether this particular NTPase is involved in ribosomal RNP export is not clear, although it is interesting that WGA was found to inhibit the ATP-dependent release of RNP from isolated nuclei (6) while we found WGA prevented ribosome export in vivo (see below). The exact role of ATP hydrolysis sponsored by this or any other nuclear NTPase is not known. Possibly ATP hydrolysis mediates cyclic conformational changes in an NPC translocator, causing a bound RNP complex to be exchanged from the nuclear to the cytoplasmic side in a manner analogous to the hydrolysis of ATP that restores DNA gyrase to an active conformation after a DNA movement event (53). If so, by analogy, it might be expected that a nonhydrolyzable ATP

analogue might suffice in RNA export albeit allowing only one translocation event per NPC. This possibility can be tested in our in vivo export assay. It has also been proposed that ATP hydrolysis might impel an actomyosin-type contractile apparatus that causes expansion of the NPC channel (37). ATP consumption may also reflect movement of RNP complexes along the nuclear matrix en route to the NPC (2); while we have no information concerning filamentous attachment of injected subunits, we would imagine if such an association occurred it might be restricted to later stages of RNP export, since the subunits we injected should be free of early assembly steps. For this reason and others (see below) we view our assay as reflecting principally the terminal event(s) in RNP export, that is, recognition by the transport apparatus (which could include proteins that deliver the RNP's to the NPC) and translocation through the NPC into the cytoplasm.

Recently it has become clear that the NPC contains a number of glycoproteins among which is a family of proteins bearing O-linked GlcNAc residues (17, 27, 34, 35, 50). These proteins are found on both the nucleoplasmic and cytoplasmic faces of the NPC. A number of studies have shown that the GlcNAc-specific lectin wheat germ agglutinin can inhibit nuclear import of proteins in vivo and in vitro (16, 22, 27, 57, 59). Apparently inhibition is not due to steric occlusion of the NPC channel by bound lectin, since small molecules and dextrans continue to diffuse through the NPC in the presence of WGA; similarly, diffusion across the NPC continues when NPCs are preassembled without WGA-binding proteins, although the NPCs lacking the WGA-binding proteins failed to carry out active transport (26). Thus, WGA binding appears to inactivate the translocation apparatus responsible for facilitated uptake of nuclear proteins. In this report we have shown that WGA diminished export of nuclear injected ribosomal subunits, suggesting that transport in our assay was an NPC process. It should be pointed out, however, that the NPC is not the only nuclear source of WGA binding proteins, although non-NPC WGA-binding proteins have not been shown to be required for any form of nuclear transport. At the very least, our results show a requirement for some glycoprotein(s) in RNP export.

Although lectin binding sites occur on both faces of the NPC, ribosome export was inhibited only when WGA was injected into the nucleus. Assuming WGA blocked export of injected subunits by interaction with the NPC, the monofaceted inhibition we observed was consistent with the work of Yoneda et al. who showed that cytoplasmically injected WGA inhibited the nuclear import of two karyophilic proteins but failed to inhibit the appearance of newly synthesized RNA in the cytoplasm (59). On the other hand, Featherstone et al. described a particular monoclonal antibody RL1 which bound a series of NPC proteins (22). RL1 inhibited nuclear protein import in Xenopus oocytes in a manner analogous to WGA, which was by inactivating the transport apparatus rather than by steric blockage. Furthermore, the appearance of 5S rRNA in the cytoplasm was inhibited to the same extent when RL1 was injected either into the nucleus or into the cytoplasm. RL1 appears to bind to the same glycosylated proteins that are bound by WGA (22), so it is not clear why RL1 inhibited RNA export when injected into either compartment but WGA inhibited export only when injected into the nucleus.

A key finding described in this report is the demonstration that prokaryotic ribosomal subunits and subunits from yeast were exported from Xenopus oocyte nuclei as efficiently as Xenopus subunits; indeed, the maximum rate for E. coli subunit export was nearly 18 times greater than for Xenopus subunits. As was the case for eukaryotic subunits, prokaryotic subunit export in the eukaryotic host was a saturable process and inhibited by either low temperature, ATP depletion, or WGA. These results contradict a previous report in which E. coli ribosomes did not exit the Xenopus nucleus after microinjection (30). Apparently in the injection experiments of the former study 70S E. coli ribosomes were used while we used either 30S or 50S ribosomal subunits. Although we can not explain the previous report, a difference between injection of 70S ribosomes versus individual subunits is unlikely to account for the different results, as there is ample evidence that RNP complexes as large 70S are capable of transport through the NPC (42).

In vivo ribosomes are derived from a large precursor RNP that assembles in the nucleus (33). Through a series of RNA cleavages this precursor particle eventually splits into pre-40S and 60S ribosomal subunits. Evidence suggests that preribosomal subunits become fully mature during export; particular proteins are removed and others are added to the presubunits as they emerge from the nucleus (see reference 36 for example). In this regard we underscore the fact that our assay for ribosomal subunit nuclear export used as substrates fully mature cytoplasmic ribosomal subunits rather than precursor subunits obtained from the nucleus. While preribosomal subunits conceivably may posses their own unique transport properties and it was unlikely that injected mature subunits participated in the same process of protein exchange associated with export of presubunits, we do not regard this dissimilarity as detracting from the results presented here. Rather, we view our data as supportive of the idea that, with respect to the final translocation step itself, different types of RNP complexes share a common mechanism of facilitated nuclear export.

A general mechanism underlying facilitated nuclear export is supported by our finding that ribosomal RNP's from yeast and E. coli were also efficiently transported in Xenopus oocytes. Although the overall configuration of ribosomal subunits from prokaryotes and eukaryotes is quite similar, there are nonetheless sequence and structural differences among their rRNAs and even greater differences among their ribosomal proteins. Therefore, we propose that nuclear export of large RNP complexes such as ribosomal subunits is dependent, at least in part, upon a collective property common to all such particles. This common property could be the presence of RNA nucleotides exposed on the surface of the RNP complexes, a suggestion extrapolated from the predictions of Clawson and Smuckler who showed that the amount of high energy phosphate expended by isolated nuclei was proportional to the number of nucleotides released as RNP (14). A substantial fraction (~30% based on RNase accessibility) of RNA in the ribosome is exposed in short stretches (8, 10). Similarly, Dworetzkey and Feldherr showed that colloidal gold particles coated with either tRNA, 5S RNA, or poly(A) were also transported from nuclei in a concentration-dependent, nuclear pore–mediated process (20). In that study it was not known whether proteins bound to the RNA-gold particles upon intranuclear injec-

tion, but it seems unlikely that such complexes could have duplicated the structures of naturally occurring RNPs. Thus, transport of RNA-coated gold particles again suggests that a common biochemical property, perhaps surface-exposed RNA, may play a dominant role in promoting export. Whether the NPC itself recognizes the RNPs directly or other nuclear proteins bind to RNPs to bring about NPC association remains to be determined. This is not to suggest that RNA alone acts as a "signal" for transport, since rRNA molecules prevented from forming their normal association with ribosomal proteins are degraded extremely rapidly and probably not exported (33); indeed, we find that purified rRNA injected into *Xenopus* oocyte nuclei is also not transported (data not shown). Perhaps specific transport proteins are designed to bind short nonhydrogen bonded stretches of exposed RNA in an RNP complex and then direct the complexes for export through the NPC; this type of recognition may be analogous to the activity of the chaperonin class of proteins that appear to bind exposed peptide segments of diverse sequence (28). Interestingly, Borer et al. described a major nucleolar protein that shuttles between the nucleus and cytoplasm (9); such a protein might function in RNP export.

Additional evidence for a generalized mechanism of RNP export can be construed from an elegant set of experiments by Legrain and Rosbash, who showed that an intron-containing mRNA was efficiently transported to the cytoplasm provided spliceosome assembly was prevented by mutational inactivation of either the intron junction or a spliceosome component (39). This result implies that RNA molecules not normally exported to the cytoplasm are restricted to the nucleus only because they are retained in assemblies like spliceosomes and thereby prevented from associating with the NPC. In the absence of retention an inappropriate RNA molecule (presumably in the form of an RNP complex) can be exported. Thus, particular RNA sequences or structures may be primarily important in formation of specific RNA–protein interactions; once assembled and liberated from retaining structures such as spliceosomes or nucleolar fibrils, RNP complexes may possibly be exported by "default," through a mechanism that relies on a collective biochemical property that promotes RNP interaction with the NPC and stimulates facilitated transport through the pore. Interestingly, Guddat et al. have reported that 5S rRNA is exported from *Xenopus* oocyte nuclei when bound either to a molecule of TFIIIA or to a molecule of ribosomal protein L5; export was refractory to substantial alterations in the 5S sequence, but only if one of the aforementioned proteins continued to bind to the altered 5S RNA. Guddat et al. concluded that TFIIIA and rpL5 define a new functional class of proteins involved in RNA export; however, since TFIIIA and rpL5 are different proteins that interact in different ways with 5S rRNA, the results of Guddat et al. are not inconsistent with our suggestion that RNP export depends less on specific sequences or arrangements of RNP structure and more on a collective property of RNPs (31). Our view of a generalized export mechanism would also seem at odds with results of Zasloff who showed that single nucleotide substitutions in tRNA molecules adversely affected their export to the cytoplasm (54). Perhaps tRNA export does operate by a different mechanism. Alternatively, the base substitutions may have interfered with the ability of the tRNAs to form stable RNP complexes with specific proteins (some substitutions concurrently prevented tRNA processing, con-

sistent with failure to form an RNP), which would be equally necessary for transport via a general mechanism.

In summary, we have presented evidence that intranuclear microinjection of ribosomal subunits leads to transport of the complexes to the cytoplasm in a manner consistent with a facilitated, NPC-associated process. The ability to biochemically modify the transported substrate and to deplete the nucleus of high energy phosphate should make it possible to detail further the mechanism of export of ribosomal subunits and RNP in general.

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