Export of mRNA from Microinjected Nuclei of *Xenopus laevis* Oocytes

Catherine Dargemont and Lukas C. Kühn

Institut Suisse de Recherches Expérimentales sur le Cancer, CH-1066 Epalinges, Switzerland

Abstract. Export of mRNA from the nucleus to the cytoplasm was studied in mature *Xenopus laevis* oocytes. In vitro transcribed, capped ^{32}P-labeled mRNA was microinjected into nuclei, and its appearance in the cytoplasm measured by counting radioactivity or by RNA extraction and gel electrophoresis. Both for a 5.0-kb transferrin receptor mRNA and a 2.0-kb 4F2 antigen heavy chain mRNA we found saturable transport with an apparent K_m of 3.6×10^8 molecules per oocyte nucleus. Under non-saturating conditions the half-time for mRNA export from the nucleus was ~2 min at 20°C. At higher concentrations of injected mRNA this half-time was prolonged, and the maximal transport rate was reached at $\sim 1.6 \times 10^8$ molecules/min. mRNA transport showed properties of an energy-dependent mechanism, since it was inhibited at 4°C or by ATP depletion. Co-injection of the cap dinucleotide m^{7}GpppG blocked the export effectively, suggesting a role for the cap in this process. The export was also inhibited by the pre-injection of wheat germ agglutinin. The effect of the lectin was specific and abolished by co-injection of N-acetylglucosamine. Finally, we found significant competitive inhibition in mRNA export by the presence of tRNA. Our results suggest that mRNA transport is a facilitated process which may share common steps with tRNA transport. Preliminary gel retardation experiments show that injected mRNA associates with endogenous nuclear proteins and suggest an exchange of some of the bound components during the transport to the cytoplasm.

In eukaryotic cells, primary RNA transcripts undergo several processing steps within the nucleus which generate mature RNA molecules that are selectively transported to the cytoplasm. The fundamental mechanisms underlying RNA export, however, remain poorly characterized, particularly for mRNAs. According to current knowledge, there exist both negative signals preventing export of immature mRNA and positive signals responsible for the acceptance of mRNA by the transport machinery.

The formation of complexes between primary RNA transcripts and splice components constitutes probably a major nuclear retention signal for pre-mRNA. Studies on mRNA export in yeast suggested that *cis*- and *trans*-acting mutations that prevent the formation of spliceosomes lead to an increased export of non-spliced mRNA (Legrain and Rosbash, 1989). In mammalian cells evidence for a similar mechanism has been seen in the function of HIV-1 Rev protein which binds to a *cis*-acting viral RNA sequence (Rev response element) and induces the appearance of incompletely spliced HIV-1 mRNA in the cytoplasm (Hadzopoulou-Cladaras et al., 1989; Malim et al., 1989; Chang and Sharp, 1989; Cullen and Malim, 1991; Kjems et al., 1991). The deduced hypothesis of a competition between spliceosome formation and pre-mRNA export is supported by morphological data. Components of the splicing machinery have recently been localized in discrete nuclear sites by immunocytochemistry or in vivo labeling using snRNA-specific antisense probes (Spector et al., 1991; Carmo-Fonseca et al., 1991*a*,*b*; Gall, 1991; Wang et al., 1991). Furthermore, pre-mRNA injected into nuclei of mammalian cells get similarly localized to speckles containing snRNPs or another spliceosome component, SC-35, whereas mRNA lacking intron sequences is distributed randomly throughout the nucleoplasm (Wang et al., 1991).

The cap structure m^{7}GpppG is the only positive signal identified to date which is required for mRNA migration to the cytoplasm (Hamm and Mattaj, 1990). Yet another feature shared among mRNA species, the poly(A)-tail, represents a good candidate for a positive signal, but its involvement in mRNA export remains poorly defined (Wickens and Gurdon, 1983). As a large number and variety of mature mRNA species is exported, it remains entirely open whether additional *cis*-acting sequences are relevant for mRNA transport. Certain signals might be more specific for individual mRNA species. A recent study performed with histone mRNA, which is intron-less and non-polyadenylated, reported that export of this mRNA depends on a particular 3′ end processing step (Eckner et al., 1991). Although these data greatly enrich the knowledge about histone regulation, they do not provide evidence that such a mechanism is of general importance for the export of other mRNAs.

Several studies have taken advantage of microinjection into *Xenopus* oocyte nuclei as a tool to investigate RNA export. Thus, it has been shown that export of tRNA from the

nucleus is uncoupled from processing events (Tobian et al., 1985). This transport is saturable and possibly mediated by a common carrier for many tRNA species (Zasloff, 1983). Export of rRNA associated with ribosomal proteins has also been found to be a facilitated and energy-dependent process that is likely associated with the nuclear pore complex (Khanna-Gupta and Ware, 1989; Bataillé et al., 1991). Newly transcribed nuclear 5S rRNA interacts transiently with La antigen which is then replaced either by the transcription factor IIIA or the ribosomal protein L5. These subsequent interactions allow the export of 5S-TFIIIA and 5S-L5 complexes (Guddat et al., 1990). snRNAs appear only transiently in the cytoplasm where they assemble with protein components to form U snRNPs while their cap m^7GpppG becomes trimethylated (Zeller et al., 1983; Mattaj, 1986). Export of snRNA (at least polymerase II-transcribed snRNAs) from the nucleus is facilitated by the monomethylated cap structure in the same way as for mRNA (Hamm and Mattaj, 1990). These studies suggest in addition the involvement of a nuclear cap-binding protein as one of the components in the nucleocytoplasmic transport machinery for mRNAs. However, it remains unknown whether this cap-binding protein exists and whether it would be sufficient to assure the transport process. Other proteins could also interact with mRNAs in order to facilitate the transport by functioning either as enzymes or carriers. For example it has been reported that RNP granules of Balbiani ring transcripts in *Chironomus* adopt a less compact conformation during their passage through the nuclear pore (Skoglund et al., 1983; Mehlin et al., 1988) suggesting that some specific factors might be involved in mRNA unfolding.

In the present report we describe the basic properties and kinetics of mRNA export after injection of capped mRNAs into nuclei of *Xenopus laevis* oocytes. We provide evidence for a saturable transport pathway which is not selective for the analyzed mRNA species. The process displayed characteristics of an energy-dependent mechanism since it was inhibited by depletion of nuclear ATP. mRNA transport was blocked by prior injection of wheat germ agglutinin (WGA)[1], a lectin known to inhibit active transport by binding to N-acetylglucosamine-containing proteins present in the nuclear pore complex. We confirm in our assay that the free cap structure m^7GpppG interferes with mRNA export. Finally, we found that tRNA was effective as a competitive inhibitor of mRNA export. These latter data suggest strongly that the transport pathways for different RNA species may use common steps.

Materials and Methods

Animals and Reagents

Female *Xenopus laevis* frogs were purchased from the African Xenopus Facility (Noodhoek, South Africa). Apyrase (grade VIII), WGA, and tRNA from bovine liver were from Sigma Chemie (Buchs, Switzerland). An ATP bioluminescence assay kit was purchased from Amersham (Buckinghamshire, England).

Preparation of Radiolabeled mRNA

The full-length human transferrin receptor (TfR) cDNA insert of pcD-TR1 (Kühn et al., 1984) was subcloned into pGEM-3Zf(−) (Promega Biotec, Madison, WI) in positive orientation relative to the promoter for T7 polymerase. The plasmid was linearized with BamHI. In vitro transcripts from this vector yield a 5.0-kb TfR mRNA that contains at its 3′ end an 84-nucleotide long poly(A)-tail followed by 32 nucleotides of pcD-vector sequence (Neupert et al., 1990). The full-length human 4F2 antigen heavy chain cDNA insert of pcD-4F2.A (Teixeira et al., 1987) was subcloned as a BamHI-BamHI fragment into pSP65 (Boehringer Mannheim Biochemicals, Mannheim, Germany) in positive orientation relative to the promoter for SP6 RNA polymerase. The plasmid was linearized with HindIII. The in vitro transcript of 2.0 kb contains at its 5′ end 100 bases of pcD vector sequences, and at the 3′ end a poly(A)-tail of ~120 nucleotides followed by 59 nucleotides of vector sequence.

For in vitro transcription, linearized plasmid DNA (1 μg) was incubated for 90 min at 37°C in a 20 μl transcription mixture containing 40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM DTT, 0.5 mM ATP and UTP, 0.25 mM CTP, 50 μM GTP, 0.5 mM cap dinucleotide m^7GpppG triphosphate (Boehringer Mannheim Biochemicals), 75 μg BSA, 40 U of RNasin (Promega Biotec), 40 μCi [α-^{32}P]CTP (800 Ci/mmol) (Amersham), and 15 to 20 U of T7 or SP6 polymerase. DNA was then digested by 2 μg of RNase-free DNase I (Worthington, Freehold, NJ) for 30 min at 37°C. Water was added to a final volume of 100 μl, proteins were extracted with phenol-chloroform, unincorporated nucleotides were removed with a Sephadex G-50 column and the RNA precipitated with 0.1 vol 3 M sodium acetate, pH 5.2, and 2.5 vol ethanol. In vitro transcribed mRNA was resuspended in 10 μl H_2O. RNA transcripts with 20-fold higher specific activity were generated by the same procedure, but with different nucleotide concentrations of UTP and CTP: 50 μM UTP, 50 μM CTP, 80 μCi [α-^{32}P]UTP and 80 μCi [α-^{32}P]CTP.

Intranuclear Microinjection and Oocyte Dissection

Stage VI oocytes were prepared from ovaries of *Xenopus laevis* females, defolliculated as described by Gurdon and Wickens (1983) and incubated in modified Barth's solution. 10 to 20 nl of solution containing the mRNA, together with bromophenol blue to monitor the actual site of injection, were microinjected per germinal vesicle. Oocytes were maintained at 20°C in Barth's solution and subsequently fixed at 4°C for 45–60 min in a solution consisting of 80% ethanol and 3% acetic acid (De la Pena and Zasloff, 1987). The oocytes were then dissected manually, and only nuclei with blue staining were considered for the analysis. The distribution of radioactivity in the nucleus and cytoplasm was determined for each oocyte by Cerenkov counting. In every experiment, four to eight oocytes were microinjected for each time point. The results obtained for each separate group of oocytes were averaged and considered as one value. The mean and SEM were calculated from the values obtained in distinct experiments. The calculation of transport rates was based on first-order kinetics. The time 0 (100% of RNA injected) was not taken into consideration since 15–20% leakage (measured by DNA injection) occurred within the first min after injection. In all the kinetic experiments, therefore, the first experimental time point measured was at 1 min. Approximately 20% leakage could also be inferred from regression curves of experimental values.

RNA Extraction

Injected mRNA was purified after the dissection of oocytes by homogenizing 10 oocytes/ml in a medium containing 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 1.5% SDS, 300 mM NaCl, 1.5 mg/ml proteinase K, followed by extraction with phenol (equilibrated with 10 mM Tris-HCl, pH 8, 1 mM EDTA), phenol-chloroform, and chloroform. RNA was precipitated with 0.1 vol sodium acetate, pH 5.2, and 2.5 vol ethanol, and analyzed by electrophoresis in 1.2% (wt/vol) agarose gels containing formaldehyde.

Gel Retardation Assays

Oocytes were injected with 2 ng of labeled TfR mRNA and incubated in MBS at 20°C. After different lengths of time, mRNA export was blocked by a shift to 4°C. Oocytes were then manually dissected at 4°C in 10 mM Hepes, pH 7.5, 40 mM KCl, 3 mM $MgCl_2$, 10% glycerol and protease inhibitors (aprotinin 0.5 μg/ml, leupeptin 1 μg/ml, pepstatin 0.7 μg/ml, and antipain 0.7 μg/ml). For each experimental condition, nuclei and cytoplasms from three oocytes were homogenized in dissection buffer (three nuclei in 9 μl, or three cytoplasms in 24 μl, respectively). Nuclear fractions were digested for 15 min at room temperature with 1 μg of RNase-free DNase I and 10U of RNase T_1 (Calbiochem-Behring Corp., San Diego, CA) and cytoplasmic fractions with 10 U of RNase T_1. Each fraction was incubated for another 10 min with 100 μg of heparin. Loading buffer (30

1. *Abbreviations used in this paper*: TfR, transferrin receptor; WGA, wheat germ agglutinin.

mM Tris, pH 7.5, 40% sucrose, 0.2% bromophenol blue) was added at 50% (vol/vol), and the cytoplasmic fractions were centrifuged at 12,000 rpm for 5 min to remove nonsoluble material. RNA-protein complexes were resolved in 6% non-denaturing acrylamide gels as described by Konarska and Sharp (1986).

Results

Kinetics of mRNA Transport from the Nucleus to the Cytoplasm

The aim of this study was to analyze the transport of mature mRNA from the nucleus to the cytoplasm. As a biological assay for this post-transcriptional event, we chose to inject radiolabeled processed mRNA into nuclei of *Xenopus laevis* oocytes and to analyze the kinetics of mRNA export by measuring the radioactivity in the nucleus and cytoplasm after various lengths of time. For each kinetics experiment, nuclei of at least 30 oocytes were injected with 2 ng of in vitro synthesized transferrin receptor (TfR) mRNA. These transcripts contained a 3′ termini with an 84-nucleotide-long poly(A)-tail followed by 32 nucleotides from the vector. After different times of incubation, oocytes were fixed at 4°C, a temperature at which mRNA transport does not occur (De la Pena and Zasloff, 1987; our data not shown), and manually dissected. Nuclear and cytoplasmic radioactivity was determined for each oocyte (Fig. 1 *A*). The mRNA transport displayed approximately first-order kinetics and the half-time, i.e., the time required for the export of 50% of the injected mRNA, was calculated to be 2.4 min (Fig. 1 *A*). As a control, labeled DNA was also injected into nuclei. Only a small fraction of this radioactivity (15–20%) was subsequently found in the cytoplasm indicating that 80 to 85% of the total injected material is in the nucleus at the beginning of each experiment.

To verify that the radioactivity appearing in the cytoplasm was not due to degraded TfR transcripts, RNA was reextracted from pooled nuclear and cytoplasmic fractions of four oocytes and analyzed by gel electrophoresis. Upon ethanol precipitation, supernatants of the RNA-containing pellets did not show any radioactivity indicating the absence of free nucleotides. Moreover, repurified injected RNA displayed the same electrophoretic properties as prior to injection (Fig. 1 *B*). This result allowed us to consider the radioactivity content as a reliable measure for the intracellular distribution of intact mRNA.

mRNA Export Is Saturable

To determine whether mRNA export occurred by diffusion or a facilitated process, increasing amounts of labeled TfR mRNA were injected into nuclei and the kinetics of transport measured. The half-time transport for each concentration is shown in Table I. From these values, it was possible to deduce the rate of mRNA transport (mRNA molecules transported per min) as a function of the number of molecules injected per nucleus (Fig. 2). The rate increased with increasing amounts of injected mRNA, but reached a maximum at ~1.6×10^8 molecules/min per oocyte. These results show that mRNA transport from the nucleus to the cytoplasm is a saturable process and exclude the hypothesis of simple diffusion through nuclear pores. The apparent K_m for the mRNA export can be estimated to be 3.6×10^8 molecules per nucleus (1 ng of TfR mRNA/nucleus).

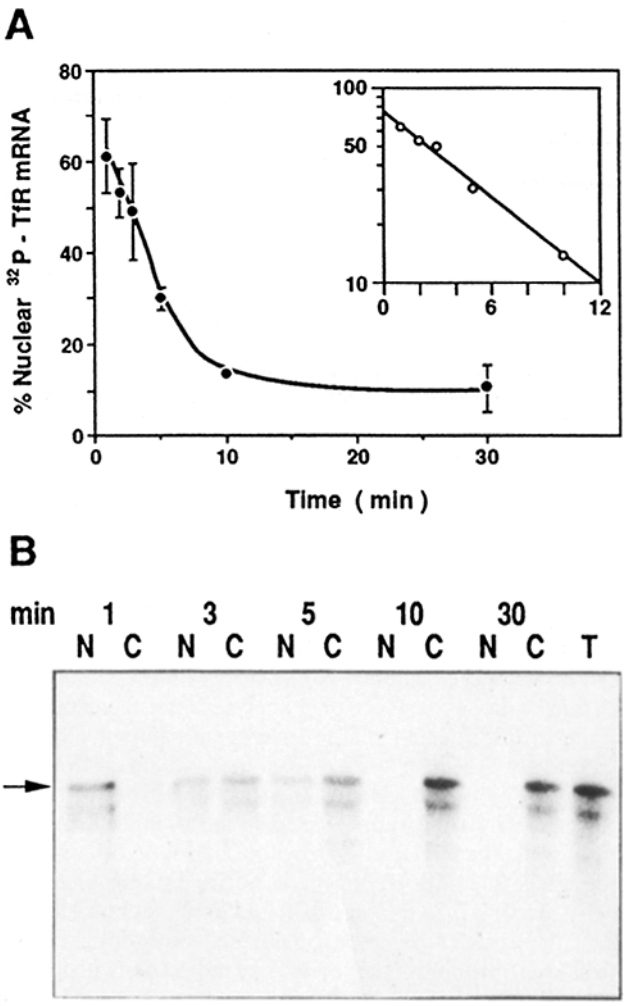

Figure 1. Kinetics of TfR mRNA export from the nucleus. 2 ng of ^{32}P-labeled TfR mRNA were injected into the nucleus of *Xenopus* oocytes. At different times after injection (1, 2, 3, 5, 10, and 30 min) oocytes were fixed in 3% acetic acid, 80% ethanol at 4°C, and manually dissected. (*A*) The radioactivity present in the nucleus and cytoplasm of each single oocyte was determined, and the percentage of radioactivity present in the nucleus calculated. Each point represents the average of three experiments, and in each experiment four to eight oocytes were injected per condition. Both linear and semilogarithmic representations are shown. (*B*) TfR mRNA re-extracted from injected oocytes after fixation and dissection was analyzed on a 1.2% agarose gel. *N*, nuclear fraction; *C*, cytoplasmic fraction; *T*, total oocytes without dissection. The arrow indicates the 5.0-kb position of TfR mRNA.

mRNA Export Involves Recognition Steps Which Are Not Sequence Specific

To further demonstrate that mRNA export occurs by a facilitated process, we tested the ability of non-labeled TfR mRNA to compete transport of labeled TfR mRNA. We inject a 30-fold excess of non-labeled TfR mRNA together with 0.4 ng of ^{32}P-labeled TfR mRNA (Fig. 3). The export of the labeled molecules was greatly retarded with a half-time of 18.7 min. However, the rate of total molecules transported per min corresponded to the theoretical one predicted from Fig. 2. This result shows that export of TfR mRNA oc-

Table I. mRNA Export Rate as a Function of the Amount of mRNA Injected Per Nucleus

TfR mRNA	Nuclear export
ng injected	$t_{1/2}$ *in min*
0.2	1.6
0.4	1.6
1.4	1.9
2.0	2.4
3.0	3.2
4.0	4.5
12.5	18.7

Oocytes were injected with increasing amounts of ^{32}P-labeled TfR mRNA. At different times after injection, oocytes were fixed and manually dissected as described in Fig. 1. The percent of total radioactivity found in the nucleus was determined for each individual oocyte. Three experiments were performed with each mRNA concentration and three to eight oocytes weere injected for each time point. The $t_{1/2}$ values for the export kinetics at different RNA concentrations were calculated from semilogarithmic representations of experimental values by linear regression.

curs in the same manner for labeled and non-labeled molecules and may involve rate-limiting recognition steps.

We then checked whether any selectivity was conferred by a defined mRNA sequence. We analyzed first the export of another transcript that shares no sequence homology with TfR mRNA: the 2.0-kb 4F2 antigen heavy chain mRNA. As shown in Fig. 4, the kinetics of export at 3.6×10^8 molecules injected (0.4 ng) appeared to be the same for 4F2 antigen mRNA as for TfR mRNA with a half-time of 2.2 min. It suggests that mRNA length is not a limiting factor in mRNA export. Injection of an 18-fold excess of non-labeled 4F2 antigen mRNA together with labeled transcript gave rise to a slower transport of the radioactive molecules with a half-time of ~20 min (Fig. 4). The transport rate deduced from these data was 1.7×10^8 molecules/min, almost the same as the value observed for TfR mRNA (Fig. 2). Identical results were obtained with non-labeled TfR mRNA as a competitor instead of 4F2 antigen mRNA (Fig. 4). These experiments demonstrate clearly that mRNA export does not involve selectivity for either of the two mRNA sequences analyzed.

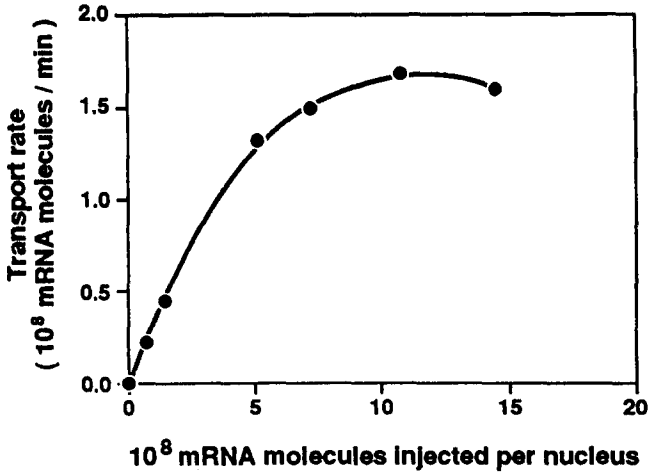

Figure 2. mRNA export is a saturable process. The rates of transport were calculated from Table I assuming first-order exponential kinetics and plotted as a function of the amount of ^{32}P-TfR mRNA injected into nuclei.

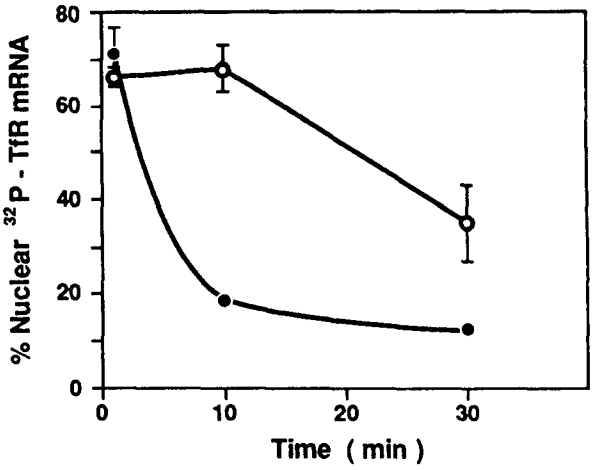

Figure 3. mRNA export involves limiting recognition steps. 0.4 ng of ^{32}P-labeled TfR mRNA were injected into oocyte nuclei in the absence (●) or in the presence (○) of a 30-fold excess of non-labeled TfR mRNA. After various time periods (1, 10, and 30 min), oocytes were fixed and dissected, and the percent of total radioactivity in the nucleus of individual oocytes determined. Each point corresponds to the average of three experiments with four to eight oocytes for each time point. For some conditions the SEM were too small to be visible on the graph.

mRNA Export Is ATP Dependent

Both the observation that no transport occurs at 4°C and that mRNA exceeds the size of the nuclear pore complex channel suggest a facilitated process requiring energy. Furthermore, nuclear protein import (Newmeyer and Forbes, 1988; Richardson et al., 1988), in vitro efflux of RNP from isolated nuclei (Agutter, 1984) and export of ribosomal subunits

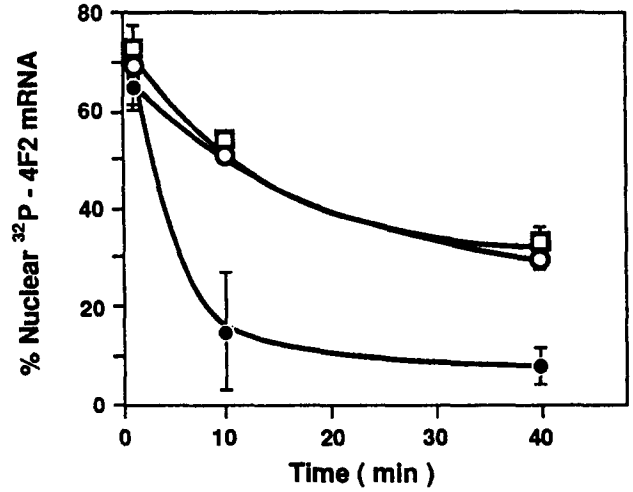

Figure 4. The process of mRNA export is not selective for a given mRNA. 0.4 ng of ^{32}P-labeled 4F2 antigen mRNA were injected into oocyte nuclei alone (●), with an 18-fold excess of non-labeled 4F2 antigen mRNA (○) or with an 18-fold excess of non-labeled TfR mRNA (□). The oocytes were incubated 1, 10, or 30 min. Each point corresponds to the average of three distinct experiments with four to eight oocytes per condition.

(Bataillé et al., 1990) occur in an ATP-dependent manner. We therefore investigated the ATP requirement in our in vivo mRNA export assay. We tested the effect of nuclear ATP depletion according to the protocol for *Xenopus* oocytes described by Bataillé et al. (1990). These authors have shown that injection of ATPase/ADPase apyrase at a final concentration of 20 U/ml (20 nl/nucleus) results in a rapid decline of the ATP concentration in the nucleus. This decrease occurs within 30 min, and the nuclear ATP depletion remains stable for at least one hour after injection. Taking into account the rapid kinetics of mRNA export, we injected apyrase into the nucleus 30 min before the injection of 2 ng of TfR mRNA. We verified by a luciferase assay that this treatment decreased ATP in the nucleus but did not affect its cytoplasmic concentration (data not shown). As a control, we injected the same volume of water 30 min before the injection of mRNA. A strong inhibition of mRNA export was observed in ATP-depleted nuclei (Fig. 5). The rate of transport was reduced from 1.6×10^8 molecules/min in the control to 0.12×10^8 molecules/min. Apyrase did not affect the transcripts themselves since TfR mRNA mixed with apyrase, reprecipitated by ethanol, and then injected was exported like mRNA without any treatment (data not shown). These results indicate that mRNA export is not only a facilitated but also an energy-dependent process.

mRNA Export Is Blocked by Wheat Germ Agglutinin

Some nuclear pore proteins carrying O-linked glycosides appear to play a major role in nuclear transport. Nuclear protein import is inhibited by antibodies against these proteins as well as by WGA, a lectin specific for N-acetylglucosamine residues (Finlay et al., 1987; Yoneda et al., 1987; Featherstone et al., 1988; Wolff et al., 1988). WGA also inhibits nuclear import of U6 snRNA (an RNA transcribed by polymerase III; Fischer et al., 1991) and export of ribosomal subunits (Bataillé et al., 1990). However this lectin has no effect on the import of polymerase II-transcribed U snRNAs (Fischer et al., 1991) and does not disturb the diffusion of small molecules through nuclear pores. It appears from these data that WGA does not affect nuclear transport by simple steric hindrance but might inhibit some specific transport steps.

These experiments prompted us to investigate the effect of WGA on mRNA export. For the export of ribosomal subunits it has been shown that WGA affected transport only when injected into the nucleus and not into the cytoplasm of *Xenopus* oocytes and that the effect was maximal when WGA was injected 1 h before the ribosomes (Bataillé et al., 1990). Since mRNA export occurs in the same direction as export of ribosomal subunits, we used an identical protocol. 2 ng of TfR mRNA were injected into oocyte nuclei that had been injected 1 h before with either WGA at a final nuclear concentration of 0.5 mg/ml, or a mixture of WGA (at the same concentration) and 50 mM GlcNAc. As a control, nuclei were also injected with mRNA alone. The results of Fig. 6 show that WGA was clearly able to inhibit mRNA export. Under this condition the rate of transport was $\sim 0.17 \times 10^8$ molecules/min, whereas $\sim 1.2 \times 10^8$ molecules/min were exported when mRNA was injected alone or after preinjection of WGA together with GlcNAc. Abolishing the WGA-induced inhibition of export by GlcNAc excludes the possibility that the results represent an irrelevant side effect of WGA. The observation that such an inhibition was strong but not complete might be a result of the limited concentration of injected WGA as compared to the number of its endogenous binding sites.

mRNA Export Is Inhibited by the Cap Dinucleotide m⁷GpppG

Mattaj and colleagues have recently reported that the cap dinucleotide m⁷GpppG is a likely signal for transport of polymerase II-transcribed RNA from the nucleus (Hamm and Mattaj, 1990). Co-injection of 50 mM m⁷GpppG (but not 5 mM) together with the U1 gene inhibited the export of newly synthesized U1 snRNA. Moreover, the transport of mRNA carrying a trimethylated cap structure was strongly delayed. Here, we investigated further the function of the cap structure in testing the ability of free m⁷GpppG to inhibit the export of mRNA in our in vivo assay which measures ex-

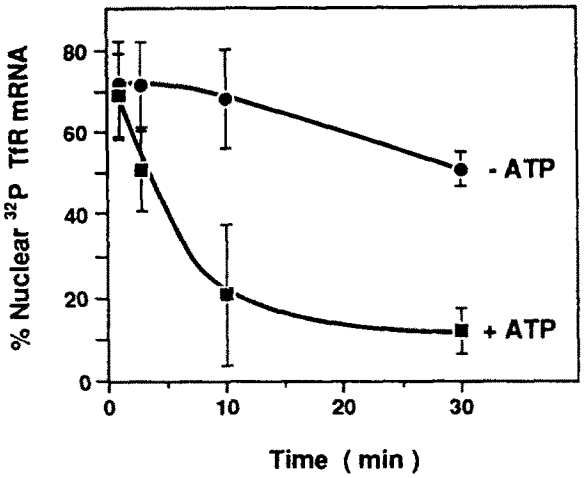

Figure 5. mRNA export is ATP-dependent. 2 ng of ^{32}P-TfR mRNA were injected into oocyte nuclei that had been preinjected 30 min before with apyrase (20 U/ml; 20 nl/nucleus) (●) or with water (■). The oocytes were incubated for 1, 3, 10, or 30 min. Each point corresponds to the average of four experiments.

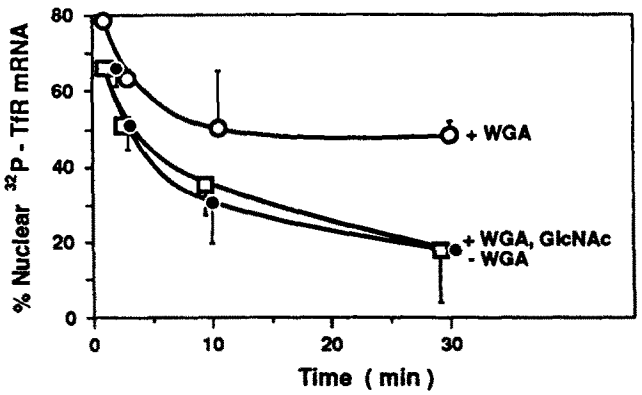

Figure 6. Effect of WGA on mRNA export. 2 ng of ^{32}P-labeled TfR mRNA were injected into oocyte nuclei that had been preinjected 1 h before with H_2O (●), with WGA (at a final concentration of 0.5 mg/ml) (○), or with WGA plus GlcNAc (50 mM final concentration) (□). Oocytes were incubated for 1, 3, 10, or 30 min at 20°C. Each point corresponds to the average of three experiments.

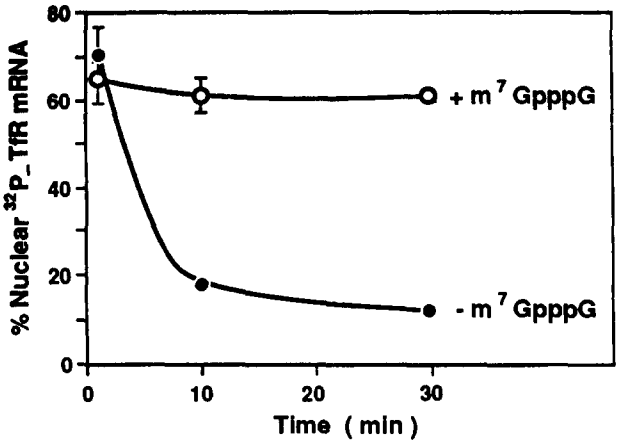

Figure 7. mRNA export is inhibited by free cap dinucleotide m^{7}GpppG. 0.4 ng of ^{32}P-labeled TfR mRNA were injected in the absence (●) or the presence (○) of 1 mM m^{7}GpppG. Incubation of oocytes was for 1, 10, or 30 min. Each point corresponds to the average of three experiments.

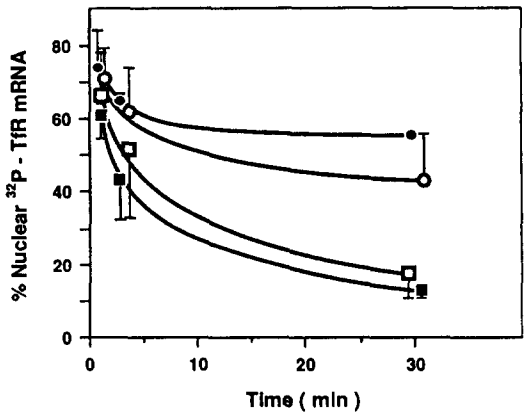

Figure 8. Effect of tRNA on mRNA export. 1 ng (●, ■) or 2 ng (○, □) radiolabeled TfR mRNA were injected into oocyte nuclei in the absence (□, ■) or in the presence (○, ●) of 40 ng tRNA from bovine liver. The oocytes were incubated for 1, 3, or 30 min. Each point corresponds to the average of three (for 1 ng) or five (for 2 ng) experiments.

clusively transport in the absence of transcription or splicing processes. As shown in Fig. 7, the injection of 1 mM m^{7}GpppG cap dinucleotide together with 0.4 ng TfR mRNA gave rise to a strong inhibition of export. Indeed, 60% of the injected mRNA remained in the nucleus after 30 min, whereas 90% were found to be cytoplasmic under control conditions. This effect was dose dependent since m^{7}GpppG at 0.1 mM did not affect the export of TfR mRNA (data not shown). It is noteworthy that the cap dinucleotide concentration active in these experiments was lower than in those previously described (Hamm and Mattaj, 1990). The explanation might reside in the length of incubation: in our transport assay mRNA export occurs within 30 min, whereas in the previous study export of transcripts was analyzed 6 h after injection of DNA (Hamm and Mattaj, 1990). During this period cap dinucleotide might diffuse through nuclear pores or be metabolized.

tRNA Is a Competitive Inhibitor of mRNA Export

Nuclear export of tRNA has been reported as a carrier-mediated and temperature-dependent process in *Xenopus* oocytes (Zasloff, 1983). It seemed, therefore, interesting to determine whether transport of mRNA and tRNA share common steps. To investigate this point we injected tRNA at a concentration at which the tRNA transport machinery is saturated and checked its effects on the export of TfR mRNA. As shown in Fig. 8, the co-injection of 40 ng of tRNA from bovine liver together with 1 or 2 ng of TfR mRNA greatly decreased the rate of appearance of TfR mRNA in the cytoplasm. The transport rates calculated from these experiments were 1.2×10^8 molecules/min and 0.23×10^8 molecules/min with 2 ng of TfR mRNA in the absence or the presence of tRNA, respectively. With 1 ng of TfR mRNA, the rate was reduced from 0.9×10^8 molecules/min in the control to 0.05×10^8 molecules/min in the presence of tRNA. It should be noticed that the tRNA-induced inhibition of mRNA export is more important at 1 ng TfR mRNA (50% saturation) than at 2 ng (75% saturation). This implies that tRNA acts as an effective competitive inhibitor of mRNA export. In other words, these two RNA species appear to use at least one common step along their transport pathway.

Analysis of Nuclear and Cytoplasmic mRNA–Protein Complexes

The present data suggest the involvement of RNA-associated proteins in mRNA transport. Such proteins constitute likely targets in WGA or cap structure-mediated inhibition of RNA export. It was of interest, therefore, to check whether injected mRNA interacts with endogenous proteins. To approach this question, oocyte nuclei were injected with ^{32}P-labeled TfR mRNA of high specific activity, and bound proteins were analyzed at different time points by a gel retardation assay. Nuclei and cytoplasms were separated and homogenized under conditions preventing the dissociation of RNA–protein complexes. These extracts were submitted to electrophoresis in non-denaturing gels after digestion with RNase T_1 (Fig. 9 *a*). Several TfR mRNA fragments were found to be resistant to RNAse T_1. It is inferred that these fragments were protected by proteins since they were not detectable after treatment with proteinase K or phenol extraction (data not shown). The patterns of these RNA bandshifts were different in nuclear and cytoplasmic fractions suggesting that some of the RNA-binding proteins have a distinct subcellular localization. Between different time points there was a change in the ratio of mRNA–protein complexes in the nucleus versus cytoplasm, that correlated with the rate of TfR mRNA export (Fig. 1). Taken together, these results suggest a dynamics in the association of proteins with mRNA along the transport pathway.

We also analyzed the RNA–protein interactions under conditions shown to affect mRNA export: the competition with an excess of non-labeled TfR mRNA (Fig. 9 *b*), ATP depletion (Fig. 9 *c*), the co-injection of m^{7}GpppG (Fig. 9 *d*), or prior injection of WGA (Fig. 9 *e*). In all these cases, RNA–protein complexes remained easily detectable in the nucleus, but their appearance in the cytoplasm was strongly

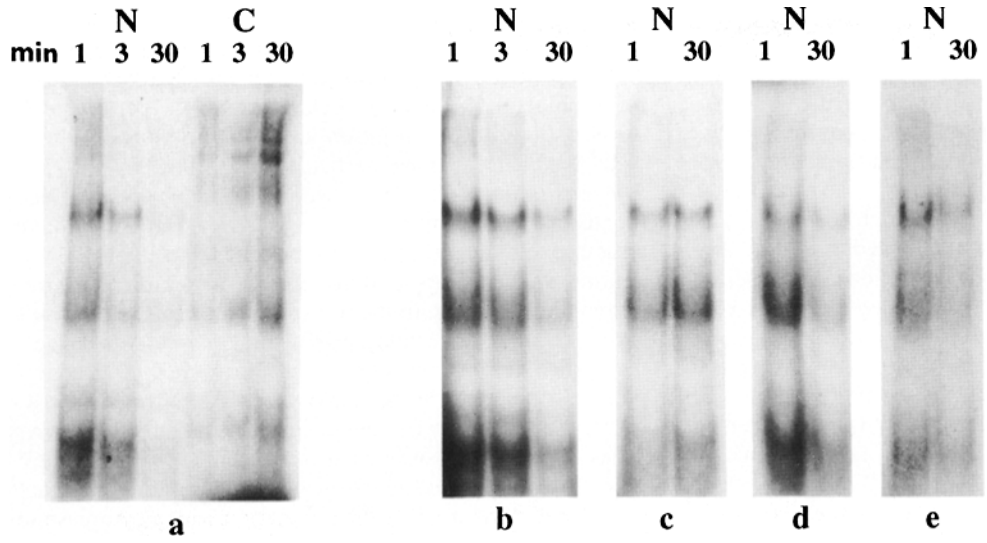

Figure 9. Analysis of TfR mRNA-protein complexes by a gel retardation assay. 2 ng of ^{32}P-labeled TfR mRNA were injected into the nucleus of *Xenopus* oocytes alone (*a*), together with a 15-fold excess of non-labeled TfR mRNA (*b*), after ATP depletion (*c*), together with 1 mM free cap dinucleotide m^{7}GpppG (*d*) or 1 h after nuclear injection of WGA (*e*). Living oocytes were dissected at different time points after injection. Nuclear and cytoplasmic fractions were homogenized, digested with RNase T_1 and RNA-protein complexes analyzed on 6% acrylamide nondenaturing gels. *N*, nuclear fraction; *C*, cytoplasmic fraction.

retarded (not shown). Retained nuclear mRNA showed an association pattern with proteins that was identical to the one obtained without inhibitor. This indicates that the formation of RNA–protein complexes observed by our method was not affected by the compounds preventing transport. Since the pattern of detectable complexes was not altered in the presence of a 15-fold excess of non-labeled mRNA, identified protein species are probably in excess over the amount of mRNA.

Discussion

The aim of this project was to describe the general properties of mRNA export from nuclei of *Xenopus laevis* oocytes. Experiments were performed by microinjection of capped and spliced mRNA into nuclei and should consequently reflect terminal events in nucleocytoplasmic RNA transport. This biological assay has previously been used to study the export of tRNA (Zasloff, 1983) and ribosomal subunits (Bataillé et al., 1990), and appears to reveal true physiological parameters. We established in control injection experiments with DNA that leakage (from the nucleus or the needle) to the cytoplasm occurs only immediately after injection and that it accounts for ~15–20% of the introduced material. We also show that injected mRNA is not significantly degraded in oocytes during the course of experiments. For the first time we report here the kinetic parameters of mRNA export from microinjected nuclei of *Xenopus* oocytes. The half time of residency of injected mRNA in these nuclei was <2 min, provided the RNA was injected at a low non-saturating concentration. Within 30 min, 90% of the mRNA reached the cytoplasm. These findings extend results obtained by Hamm and Mattaj (1990) who reported that the maximal time necessary for splicing and export of an intron-containing mRNA was 90 min, of which 60 to 70 min were attributed to the splice reactions. Thus, according to these authors, mature mRNAs are transported within 20 to 30 min in oocytes. The fast kinetics observed here may reflect the extraordinary high density of nuclear pores in *Xenopus laevis* oocytes with 60 pores/μm^2 (Gerace and Burke, 1988), i.e., ~10^8 pores/nucleus.

The export of mRNA was saturable indicating that it occurred by a facilitated process rather than by passive diffusion. Its rate reached a maximum of 1.6×10^8 molecules/min both for TfR and 4F2 antigen mRNA that differ about threefold in size. This value is 10-fold lower than the transport rate of tRNA (19×10^8 molecules/min; Zasloff, 1983), but similar to those reported for ribosomal subunits ($0.17–3 \times 10^8$ subunits/min; Bataillé et al., 1990). Taking into account that mRNA molecules are larger than the size of the nuclear pore channel and that RNA-coated gold particles are translocated through nuclear pores of oocytes (Dworetzky and Feldherr, 1988), facilitated transport mechanisms are expected to govern mRNA export. We demonstrate that mRNA transport involves, indeed, some rate-limiting recognition steps by showing the ability of a non-labeled mRNA to compete its labeled homolog. The half-maximal rate of transport (apparent K_m) was reached at a concentration of 3.6×10^8 mRNA molecules (1 ng TfR mRNA) injected per nucleus. It is worth noting that this value reflects only the behaviour of injected mRNA. Indeed, it is not possible to consider the amount of injected mRNA as negligible when compared to endogenous mRNA. Stage VI oocytes contain ~80 ng poly(A)$^+$RNA, of which 1/10 is nuclear (Dolecki and Smith, 1979; Gurdon and Wickens, 1983). Steady state poly(A)$^+$ RNA synthesis was estimated in the range of 8–10 ng/day per oocyte taking into account an average size of 2.5 kb (Dolecki and Smith, 1979). 80% of this RNA is however short lived and may represent hnRNA which is not exported to the cytoplasm.

mRNA export is not only facilitated but also displays properties of an energy-dependent process since it is fully inhibited by lowering the temperature to 4°C or by depleting ATP from nuclei. It has been reported that nuclear protein import (Newmeyer and Forbes, 1988; Richardson et al., 1988) as well as ribosomal subunits export (Bataillé et al., 1990) also require metabolic energy in vivo. The import of cytoplasmic proteins into the nucleus occurs in two distinct

steps: binding to the pore via a nuclear localization signal, and translocation through the pore channel which depends on ATP hydrolysis (Newmeyer and Forbes, 1988; Richardson et al., 1988; reviewed by Goldfarb and Michaud, 1991). It remains unclear whether ATP is necessary for the dissociation of the translocated protein from the signal sequence receptor, for the pore dilation or the movement through the pore. In other words, ATP may be required for the functioning of the transport machinery and/or for a modification of transported molecules themselves. Although the site of ATP hydrolysis has not been definitely established, it is generally assumed that the transport energy is provided by a nuclear envelope NTPase (Agutter, 1984). In analogy to translation and splicing where ATP-dependent RNA helicases have been proposed to be involved (Ray et al., 1985; Abramson et al., 1987; Dalbadie-McFarland and Abelson, 1990; Burgess et al., 1990; Company et al., 1991), one may wonder whether ATP-dependent RNA export is also facilitated by an unwindase.

A family of proteins bearing O-linked N-acetylglucosamine (GlcNAc) residues has recently been found to be essential for the formation of functional nuclear pores. The importance of these glycoproteins to nuclear transport has been demonstrated by using either a monoclonal antibody directed against one of the N-acetylglucosamine-peptide epitopes (Featherstone et al., 1988) of by WGA, a lectin specific for GlcNAc residues. When injected into the cytoplasm in vivo, both these reagents block protein transport to the nucleus and more precisely the ATP-dependent translocation through the nuclear pore (Finlay et al., 1987; Yoneda et al., 1987; Dabauvalle et al., 1988; Newmeyer and Forbes, 1988; Wolff et al., 1988). In addition to preventing protein import, WGA is also known to inhibit the nuclear export of ribosomal subunits (Bataillé et al., 1990) and the import of U6 snRNA (Fischer et al., 1991). In the present study, we find now that the lectin is in addition able to block mRNA export. WGA does not appear to prevent nuclear transport by physically occluding the nuclear pore channel, since diffusion (Finlay et al., 1987; Yoneda et al., 1987; Dabauvalle et al., 1988) as well as specific transport of U1 and U5 snRNAs (Fischer et al., 1991) still occur. In addition, nuclei that lack specific WGA-binding proteins cannot import protein anymore, but remain functional for diffusion. This selective transport competence is restored by addition of specific GlcNAc-bearing pore proteins (Finlay and Forbes, 1990). Some of these proteins have been characterized and the most abundant of them, p62, has been cloned; however, its sequence has failed to help the understanding of the p62 function in nuclear transport (D'Onofrio et al., 1988; Starr et al., 1990). Recently it has been reported that three of these proteins, p62, p58, and p54 define a complex with strong intramolecular interactions that is essential for pore function (Finlay et al., 1991). However, some other types of nuclear protein such as transcription factors or chromatin proteins also bear GlcNAc residues (Jackson and Tjian, 1988; Kelly and Hart, 1989). Although none of these proteins has been shown to play a role in nuclear transport, we cannot formally exclude that inhibition by WGA could be mediated by a non-pore protein.

Our in vivo assay allowed us to study whether *cis*-acting signals are involved in terminal events of RNA export. The comparison of two mRNAs that differ in size and sequence did not indicate any divergence in their transport rates and pathways, since competition between the two mRNAs was as efficient with heterologous as with homologous transcripts. These data support the idea that mRNA transport is not selective for a given mRNA sequence. This conclusion seemingly contradicts a recent report in which 3′ end processing of specific sequences in histone mRNA were shown to stimulate RNA export (Eckner et al., 1991). Histone mRNA, however, is an intron-less and non-polyadenylated mRNA which may have particular properties.

Positive signals for mRNA export are likely to be provided by structures present in most mRNAs: the m^7GpppG cap structure or the poly(A)-tail. In showing a quantitative competition with m^7GpppG dinucleotide, we confirm the requirement of the cap in the transport of polymerase II transcripts (Hamm and Mattaj, 1990). Both sets of data suggest the involvement of a nuclear cap-binding protein(s) as an important component in the nucleocytoplasmic transport machinery for mRNAs. For the m^7GpppG-capped U6 snRNA, it has recently been proposed that the m^7GpppG structure may also represent a general cytoplasmic retention signal which mediates probably the interaction with a cytoplasmic cap-binding protein (Fischer et al., 1991). In this case coinjection of free m^7GpppG into the cytoplasm of *Xenopus laevis* oocytes induced the nuclear import of m^7GpppG-capped U6 snRNA, presumably by competing RNA retention. Although the best characterized cap-binding proteins are cytoplasmic and involved in translation initiation (Shatkin, 1985), nuclear cap-binding proteins have also been described (Patzelt et al., 1983). Their identification should allow to determine whether nuclear and cytoplasmic cap-binding proteins are related or even identical. Two hypotheses have been proposed for the role of a cap-binding protein in RNA export (Hamm and Mattaj, 1990): it could represent an RNA carrier that shuttles between the nucleus and cytoplasm, or it may mediate the interaction between the RNA and the transport machinery. We report in this study that tRNA acts like a competitive inhibitor of mRNA export which may imply a common step along the transport pathways of these two RNA species. Since tRNA is not capped, it would seem that the competition occurs at a step that is distinct of the cap structure recognition. This could mean that the cap is necessary but not sufficient for mRNA export.

In conclusion, it is becoming increasingly apparent that nucleocytoplasmic transport mechanisms of different RNA species share common properties. Indeed, the export of tRNA, mRNA, and ribosomal subunits are all saturable, occur by facilitated processes that are temperature sensitive, and require energy. In all cases RNA export seems to involve RNA-protein interactions. The identification of the specific RNA-binding proteins and the dynamic properties of the formation and dissociation of such RNA-protein complexes will undoubtedly provide a key to the understanding of RNA export. In the present study we have initiated the analysis of such RNA-protein interactions. Our data indicate clearly the association of endogenous proteins with micro-injected mRNA. RNA-protein complexes appeared to be different in the nucleus and cytoplasm suggesting a successive exchange of bound proteins along the export pathway. It remains an open question whether these RNA-protein interactions are directly involved in the mRNA transport. Further refinement of the analytical tools should allow us to characterize the observed proteins and define their function in mRNA export from the nucleus.

The authors thank P. Wellauer and O. Hagenbüchle for advice in setting up *Xenopus* oocyte microinjection, as well as J. Salamero, E. Nigg, and B. Henderson for carefully reading the manuscript.

The work was supported by the Swiss National Science Foundation, and C. Dargemont is a recipient of a fellowship from the European Molecular Biology Organization.

Received for publication 3 February 1992 and in revised form 31 March 1992.

References

Abramson, R. D., T. E. Dever, T. G. Lawson, B. K. Ray, R. E. Thach, and W. C. Merrick. 1987. The ATP-dependent interaction of eukaryotic initiation factors with mRNA. *J. Biol. Chem.* 262:3826–3832.

Agutter, P. S. 1984. Nucleocytoplasmic RNA transport. *Subcell. Biochem.* 10:281–357.

Bataillé, N., T. Helser, and H. M. Fried. 1990. Cytoplasmic transport of ribosomal subunits microinjected into the Xenopus laevis oocyte nucleus: a generalized, facilitated process. *J. Cell Biol.* 111:1571–1582.

Burgess, S., J. R. Couto, and C. Guthrie. 1990. A putative ATP binding protein influences the fidelity of branchpoint recognition in yeast splicing. *Cell.* 60:705–717.

Carmo-Fonseca, M., R. Pepperkok, B. S. Sproat, W. Ansorge, M. S. Swanson, and A. I. Lamond. 1991*a*. In vivo detection of snRNP-rich organelles in the nuclei of mammalian cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:1863–1873.

Carmo-Fonseca, M., D. Tollervey, R. Pepperkok, S. M. L. Barabino, A. Merdes, C. Brunner, P. D. Zamore, M. R. Green, E. Hurt, and A. I. Lamond. 1991*b*. Mammalian nuclei contain foci which are highly enriched in components of the pre-mRNA splicing machinery. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:195–206.

Chang, D. D., and P. A. Sharp. 1989. Regulation by HIV Rev depends upon recognition of splice sites. *Cell.* 59:789–795.

Company, M., J. Arenas, and J. Abelson. 1991. Requirement of the RNA helicase like protein PRP 22 for release of messenger RNA from sliceosomes. *Nature (Lond.).* 349:487–493.

Cullen, B. R., and M. H. Malim. 1991. The HIV-1 Rev protein: prototype of a novel class of eukaryotic post-transcriptional regulators. *Trends Biochem. Sci.* 16:346–350.

Dabauvalle, M.-C., B. Schulz, U. Scheer, and R. Peters. 1988. Inhibition of nuclear accumulation of karyophilic proteins in living cells by microinjection of the lectin wheat germ agglutinin. *Exp. Cell Res.* 174:291–296.

Dalbadie-McFarland, G., and J. Abelson. 1990. PRP 5: a helicase-like protein required for mRNA splicing in yeast. *Proc. Natl. Acad. Sci. USA.* 87:4236–4240.

De la Pena, P., and M. Zasloff. 1987. Enhancement of mRNA nuclear transport by promoter elements. *Cell.* 50:613–619.

Dolecki, G. J., and L. D. Smith. 1979. Poly(A)$^+$ RNA metabolism during oogenesis in Xenopus laevis. *Dev. Biol.* 69:217–236.

D'Onofrio, M., C. M. Starr, M. K. Park, G. D. Holt, R. S. Haltiwanger, G. W. Hart, and J. A. Hanover. 1988. Partial cDNA sequence encoding a nuclear pore protein modified by O-linked N-acetylglucosamine. *Proc. Natl. Acad. Sci. USA.* 85:9595–9599.

Dworetzky, S. I., and C. M. Feldherr. 1988. Translocation of RNA-coated gold particles through the nuclear pores of oocytes. *J. Cell Biol.* 106:575–584.

Eckner, R., W. Ellmeier, and M. L. Birnstiel. 1991. Mature mRNA 3′ end formation stimulates RNA export from the nucleus. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3513–3522.

Featherstone, C., M. K. Darby, and L. Gerace. 1988. A monoclonal antibody against the nuclear pore complex inhibits nucleocytoplasmic transport of protein and RNA in vivo. *J. Cell Biol.* 107:1289–1297.

Finlay, D. R., and D. J. Forbes. 1990. Reconstitution of biochemically altered nuclear pores: transport can be eliminated and restored. *Cell.* 60:17–29.

Finlay, D. R., D. D. Newmeyer, T. M. Price, and D. J. Forbes. 1987. Inhibition of in vitro nuclear transport by a lectin that binds to nuclear pores. *J. Cell Biol.* 104:189–200.

Finlay, D. R., E. Meier, P. Bradley, J. Horecka, and D. J. Forbes. 1991. A complex of nuclear pore proteins required for pore function. *J. Cell Biol.* 114:169–183.

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

Gall, J. G. 1991. Spliceosomes and snurposomes. *Science (Wash. DC).* 252:1499–1500.

Gerace, L., and D. Burke. 1988. Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* 4:335–374.

Goldfarb, D., and N. Michaud. 1991. Pathways for the nuclear transport of proteins and RNAs. *Trends Cell Biol.* 1:20–24.

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

Gurdon, J. B., and M. P. Wickens. 1983. The use of Xenopus oocytes for the expression of cloned genes. *Methods Enzymol.* 101:370–386.

Hazopoulou-Cladaras, M., B. K. Felber, C. Cladaras, A. Athanssopoulos, A. Tse, and G. N. Pavlakis. 1989. The rev (trs/art) protein of human immunodeficiency virus type 1 affects viral mRNA and protein expression via a cis-acting sequence in the env region. *J. Virol.* 63:1265–1274.

Hamm, J., and I. W. Mattaj. 1990. Monomethylated cap structures facilitate RNA export from the nucleus. *Cell.* 63:109–118.

Jackson, S. P., and R. Tjian. 1988. O-glycosylation of eukaryotic transcription factors: implications for mechanisms of transcriptional regulation. *Cell.* 55:125–133.

Kelly, W. G., and G. W. Hart. 1989. Glycosylation of chromosomal proteins: localization of O-linked N-acetylglucosamine in Drosophila chromatin. *Cell.* 57:243–251.

Khanna-Gupta, A., and V. C. Ware. 1989. Nucleocytoplasmic transport of ribosomes in a eukaryotic system: is there a facilitated transport process. *Proc. Natl. Acad. Sci. USA.* 86:1791–1795.

Kjems, J., A. D. Frankel, and P. A. Sharp. 1991. Specific regulation of mRNA splicing in vitro by a peptide from HIV-1 Rev. *Cell.* 67:169–178.

Konarska, M. M., and P. A. Sharp. 1986. Electrophoretic separation of complexes involved in the splicing of precursors to mRNAs. *Cell.* 46:845–855.

Kühn, L. C., A. McClelland, and F. H. Ruddle. 1984. Gene transfer, expression, and molecular cloning of the human transferrin receptor gene. *Cell.* 37:95–103.

Legrain, P., and M. Rosbash. 1989. Some cis- and trans-acting mutants for splicing target pre-mRNA to the cytoplasm. *Cell.* 57:573–583.

Malim, M. H., J. Hauber, S.-Y. Le, J. V. Maizel, and B. R. Cullen. 1989. The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. *Nature (Lond.).* 338: 254–257.

Mattaj, I. W. 1986. Cap trimethylation of U snRNA is cytoplasmic and dependent on U snRNP protein binding. *Cell.* 46:905–911.

Mehlin, H., A. Lönnroth, U. Skoglund, and B. Daneholt. 1988. Structure and transport of a specific premessenger RNP particle. *Cell Biol. Int. Rep.* 12:729–736.

Neupert, B., N. A. Thompson, C. Meyer, and L. C. Kühn. 1990. A high yield affinity purification method for specific RNA-binding proteins: isolation of the iron regulatory factor from human placenta. *Nucl. Acids Res.* 18:51–55.

Newmeyer, D. D., and D. J. Forbes. 1988. Nuclear import can be separated into distinct steps in vitro: nuclear pore binding and translocation. *Cell.* 52:641–653.

Patzelt, E., D. Blaas, and E. Kuechler. 1983. CAP binding proteins associated with the nucleus. *Nucl. Acids Res.* 11:5821–5835.

Ray, B. K., T. G. Lawson, J. C. Kramer, M. H. Cladaras, J. A. Grifo, R. D. Abramson, W. C. Merrick, and R. E. Thach. 1985. ATP-dependent unwinding of messenger RNA structure by eukaryotic initiation factors. *J. Biol. Chem.* 260:7651–7658.

Richardson, W. D., A. D. Mills, S. M. Dilworth, R. A. Laskey, and C. Dingwall. 1988. Nuclear protein migration involves two steps: rapid binding at the nuclear envelope followed by slower translocation through nuclear pores. *Cell.* 52:655–664.

Shatkin, A. J. 1985. mRNA cap binding proteins: essential factors for initiating translation. *Cell.* 40:223–224.

Skoglund, U., K. Andersson, B. Björkroth, M. M. Lamb, and B. Daneholt. 1983. Visualization of the formation and transport of a specific hnRNP particle. *Cell.* 34:847–855.

Spector, D. L., X.-D. Fu, and T. Maniatis. 1991. Associations between distinct pre-mRNA splicing components and the cell nucleus. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3467–3481.

Starr, C. M., M. D'Onofrio, M. K. Park, and J. A. Hanover. 1990. Primary sequence and heterologous expression of nuclear pore glycoprotein p62. *J. Cell Biol.* 110:1861–1871.

Teixeira, S., S. Di Grandi, and L. C. Kühn. 1987. Primary sequence of the human 4F2 antigen heavy chain predicts a transmembrane protein with a cytoplasmic N-terminus. *J. Biol. Chem.* 262:9574–9580.

Tobian, J. A., L. Drinkard, and M. Zasloff. 1985. tRNA nuclear transport: defining the critical regions of human tRNA^{met} by point mutagenesis. *Cell.* 43:415–422.

Wang, J., L.-G. Cao, Y.-L. Wang, and T. Pederson. 1991. Localization of premessenger RNA at discrete nuclear sites. *Proc. Natl. Acad. Sci. USA.* 88:7391–7395.

Wickens, M. P., and J. B. Gordon. 1983. Post-transcriptional processing of Simian virus 40 late transcripts in injected frog oocytes. *J. Mol. Biol.* 163:1–26.

Wolff, B., M.C. Willingham, and J. A. Hanover. 1988. Nuclear protein import: specificity for transport across nuclear pores. *Exp. Cell Res.* 178:318–334.

Yoneda, Y., N. Imamoto-Sonobe, M. Yamaizumi, and T. Uchida. 1987. Reversible inhibition of protein import into the nucleus by wheat germ agglutinin injected into cultured cells. *Exp. Cell Res.* 173:586–595.

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

Zeller, R., T. Nyffenegger, and E. M. De Robertis. 1983. Nucleocytoplasmic distribution of snRNPs and stockpiled snRNA-binding proteins during oogenesis and early development in Xenopus laevis. *Cell.* 32:425–434.