RNA (2000), *6*:245–256. Cambridge University Press. Printed in the USA.

Deciphering the cellular pathway for transport of poly(A)-binding protein II

ANGELO CALADO,[1] ULRIKE KUTAY,[2] UWE KÜHN,[3] ELMAR WAHLE,[3] and MARIA CARMO-FONSECA[1]

[1]Institute of Histology and Embryology, Faculty of Medicine, University of Lisbon, 1649-028 Lisboa, Portugal
[2]Zentrum für Molekulare Biologie, Universität Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany
[3]Universität Halle, Institut für Biochemie, Kurt-Mothes-Strasse 3, 06120 Halle, Germany

ABSTRACT

Poly(A)-binding protein II (PABP2) is an abundant nuclear protein that binds with high affinity to nascent poly(A) tails, stimulating their extension and controlling their length. In the cytoplasm, a distinct protein (PABP1) binds to poly(A) tails and participates in mRNA translation and stability. How cytoplasmic PABP1 substitutes for nuclear PABP2 is still unknown. Here we report that PABP2 shuttles back and forth between nucleus and cytoplasm by a carrier-mediated mechanism. A potential novel type of nuclear localization signal exists at the C-terminus of the protein, a domain that is highly enriched in methylated arginines. PABP2 binds directly to transportin in a RanGTP-sensitive manner, suggesting an involvement of this transport receptor in mediating import of the protein into the nucleus. Although PABP2 is small enough to diffuse passively through the nuclear pores, protein fusion experiments reveal the existence of a facilitated export pathway. Accordingly, no transport of PABP2 to the cytoplasm occurs at 4 °C. In contrast, export of PABP2 continues in the absence of transcription, indicating that transport to the cytoplasm is independent of mRNA traffic. Thus, rather than leaving the nucleus as a passive passenger of mRNAs, the data suggest that PABP2 interacts with the nuclear export machinery and may therefore contribute to mRNA transport.

Keywords: nucleocytoplasmic transport; PABP2; polyadenylation; transportin

INTRODUCTION

With only one known exception, all eukaryotic mRNAs are posttranscriptionally modified at their 3′ ends by addition of a poly(A) tail (Adesnik et al., 1972). Poly(A) tails are generated in the nucleus in a two-step reaction that involves cleavage of the nascent transcript and subsequent polyadenylation of the upstream RNA fragment (reviewed by Colgan & Manley, 1997; Kühn & Wahle, 1997). After transport of mature mRNAs to the cytoplasm, poly(A) tails increase the efficiency of translation initiation and help to stabilize the mRNAs (Beelman & Parker, 1995; Sachs et al., 1997). Presumably after interaction with the translational apparatus, poly(A) tails are shortened by a deadenylation mechanism that triggers the decay of the mRNA (Beelman & Parker, 1995).

The formation of poly(A) tails in the nucleus involves a number of *trans*-acting protein factors, namely cleavage and polyadenylation specificity factor (CPSF), cleavage stimulation factor (CstF), cleavage factors I_m and II_m (CF I_m and CF II_m), poly(A) polymerase (PAP), and poly(A)-binding protein (PABP). Specific RNA–protein contacts and protein–protein interactions cause the assembly of these factors with the RNA substrate, generating a multicomponent 3′-end-processing complex (reviewed by Keller, 1995; Manley, 1995; Kühn & Wahle, 1997). The precursor RNA (pre-mRNA) is first endonucleolytically cleaved at a particular phosphodiester bond, and the resulting 3′-OH group then receives approximately 250 adenylate residues. The polyadenylation reaction is catalyzed by poly(A) polymerase, but this enzyme by itself has a very low and unspecific affinity for RNA. In the 3′-end-processing complex, poly(A) polymerase must interact with CPSF to be tethered to the primer. However, poly(A) synthesis in the presence of poly(A) polymerase and CPSF is slow and inefficient. Processive and efficient polymerization requires an additional factor, the poly(A)-binding protein.

Comparison of mammalian and yeast pre-mRNA 3′-end-processing components reveals that, although many homologies can be found, the proteins are not function-

Reprint requests to: Maria Carmo-Fonseca, Institute of Histology and Embryology, Faculty of Medicine, University of Lisbon, 1649-028 Lisboa, Portugal; e-mail: hcarmo@correio.fm.ul.pt.

ally interchangeable (for a recent review, see Keller & Minvielle-Sebastia, 1997). An apparent case of divergence during evolution occurs with poly(A)-binding proteins. Yeast contains a single poly(A)-binding protein (termed Pab1p), which is an essential protein present in both the nucleus and the cytoplasm (Sachs et al., 1986). In the nucleus it stimulates polyadenylation and controls poly(A) tail length (Amrani et al., 1997; Minvielle-Sebastia et al., 1997; Brown & Sachs, 1998), whereas in the cytoplasm it is involved in translation initiation (Sachs & Davis, 1989; Sachs & Deardorff, 1992; Tarun & Sachs, 1995, 1996; Le et al., 1997) and mRNA degradation (Caponigro & Parker, 1995; Boeck et al., 1998; Coller et al., 1998). In contrast, mammalian cells contain two distinct proteins called poly(A)-binding protein I (PABP1) and poly(A)-binding protein II (PAB II or PABP2). PABP1 is predominantly detected in the cytoplasm (Görlach et al., 1994), whereas PABP2 is localized in the nucleus (Wahle, 1991; Krause et al., 1994). PABP1, which is the mammalian counterpart of yeast Pab1p (51% identity), is apparently involved in both cytoplasmic mRNA stability (Bernstein et al., 1989; Wormington et al., 1996; Afonina et al., 1997; Ford et al., 1997; Körner & Wahle, 1997) and translation (Craig et al., 1998), but to date there is no evidence that it participates in nuclear pre-mRNA 3′-end processing (see Keller & Minvielle-Sebastia, 1997). Consistent with its nuclear localization, PABP2 is the mammalian protein involved in polyadenylation. PABP2 stimulates processive poly(A) addition and controls the size of the tail to be ~250 nt in length (Wahle, 1991, 1995; Bienroth et al., 1993).

How PABP2 bound to poly(A) tails in the nucleus is replaced by PABP1 in the cytoplasm remains unknown, but recent evidence indicates that both PABP1 and PABP2 shuttle between nucleus and cytoplasm (Afonina et al., 1998; Chen et al., 1999). Because it is not known when PABP1 exchanges with PABP2, one possibility is that PABP2 crosses the nuclear pores in association with the mRNA and dissociates from the poly(A) tail in the cytoplasm. Alternatively, PABP2 may dissociate from the poly(A) tail in the nucleus and be exported to the cytoplasm independently of the mRNA. To further investigate this question, we have analyzed the cellular pathway involved in transport of PABP2 between nucleus and cytoplasm.

All traffic of RNA and protein molecules between the nucleus and the cytoplasm is routed through nuclear pore complexes. Although small molecules may cross the pores by simple diffusion, eukaryotic cells have evolved complex transport pathways mediated by saturable receptors or carriers (for a recent review, see Ohno et al., 1998). As expected from a receptor-mediated process, nucleocytoplasmic transport is energy dependent and substrate specific. Accordingly, distinct pathways involved in nuclear import and export of different classes of proteins and RNAs have been identified (reviewed by Nigg, 1997; Nakielny & Dreyfuss, 1997; Ohno et al., 1998; Stutz & Rosbash, 1998). In addition to receptors, a key player in nucleocytoplasmic transport is the small GTPase Ran. Like other G proteins, Ran switches between a GDP-bound and a GTP-bound state, depending on the activity of regulators. Ran's regulators are asymmetrically distributed in the cell, implying that the concentration of Ran-GTP is low in the cytoplasm and high in the nucleus. Because import substrates dissociate from their receptors in the presence of Ran-GTP, whereas export substrates require Ran-GTP to associate with the receptor and the interaction is disrupted upon GTP hydrolysis, the steep Ran-GTP gradient across the nuclear envelope was proposed to explain why some substrates are moved into the nucleus and others are moved out of it (Görlich et al., 1996; Görlich, 1997).

Here we show that PABP2 shuttles continuously between the nucleus and the cytoplasm making use of a facilitated carrier mechanism. PABP2 is shown to contain a nuclear localization signal (NLS) in its C-terminal domain and to bind directly to transportin in a RanGTP-sensitive manner. As transportin is a nuclear transport receptor previously shown to mediate nuclear import of shuttling hnRNP proteins (Pollard et al., 1996; Siomi et al., 1997), this finding suggests that PABP2 and hnRNP proteins share the same nuclear import pathway. Interestingly, the facilitated export of PABP2 to the cytoplasm is independent of ongoing transcription, suggesting that the protein may play a role in mRNA transport similar to that of shuttling hnRNPs.

RESULTS

PABP2 shuttles between the nucleus and the cytoplasm

Immunofluorescence microscopy using affinity-purified antibodies reveals PABP2 exclusively localized in the nucleus (Fig. 1A), as previously reported by Krause et al. (1994). Within the nucleus, PABP2 appears widespread throughout the nucleoplasm with higher concentration in "speckles" (Fig. 1A, arrows), which represent domains enriched in poly(A) RNA (Fig. 1B, arrows; see also Carter et al., 1991).

To investigate whether PABP2 is permanently retained in the nucleus or transiently shuttles to the cytoplasm, we have made use of an interspecies heterokaryon assay (Borer et al., 1989; Piñol-Roma & Dreyfuss, 1992). The migration of human PABP2 was monitored in human-*Drosophila* heterokaryons produced by polyethylene glycol-induced fusion of HeLa and SL2 cells (Fig. 1C–E). Heterokaryons were kept in culture for up to 6 h in the presence or absence of the protein synthesis inhibitor emetine. Specific identification of human PABP2 was possible because the

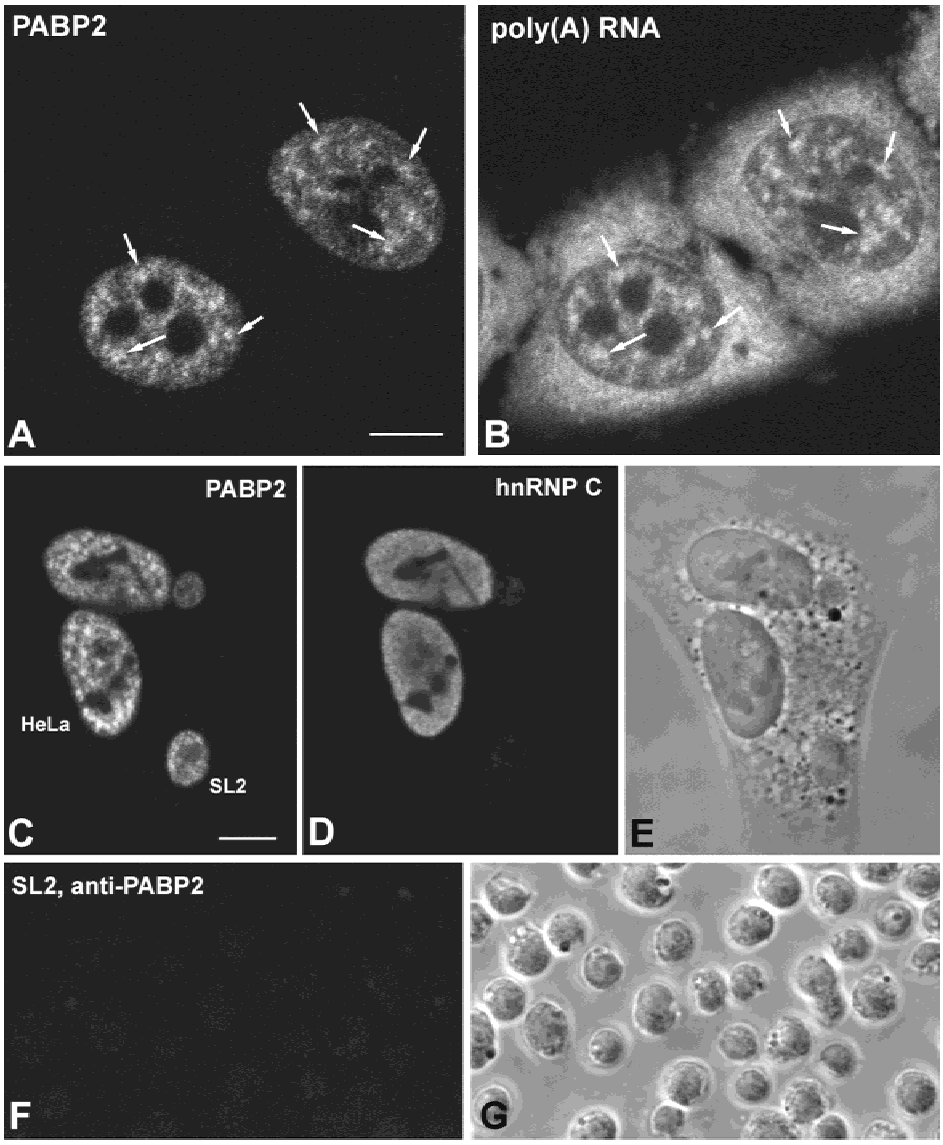


FIGURE 1. PABP2 shuttles between nucleus and cytoplasm. HeLa cells were immunostained with affinity-purified anti-PABP2 antibodies (**A**) and hybridized with a poly(U) riboprobe (**B**). PABP2 is detected exclusively in the nucleoplasm whereas poly(A) RNA is detected in both cytoplasm and nucleoplasm. Within the nucleoplasm, PABP2 and poly(A) RNA colocalize in speckles (arrows). **C–E**: HeLa cells were fused with *Drosophila* SL2 cells to form heterokaryons. HeLa cells were treated with emetine for 3 h before fusion. After fusion the cells were kept in culture for 3 h in the presence of emetine. Heterokaryons were fixed and double-labeled with affinity-purified anti-PABP2 antibodies (**C**) and monoclonal 4F4 directed against hnRNP C (**D**). **E** shows the corresponding phase-contrast image. **F**: The affinity-purified anti-PABP2 antibodies do not react with SL2 nuclei. **G** shows the corresponding phase-contrast image. Bar = 10 μm.

affinity-purified antibodies do not cross-react with *Drosophila* proteins (Fig. 1F,G and data not shown). As a control, heterokaryons were double-labeled with a monoclonal antibody specific for human hnRNP C, a protein that is always restricted to the nucleus (Piñol-Roma & Dreyfuss, 1992). In the absence of emetine, both human PABP2 and hnRNP C proteins synthesized in the cytoplasm of heterokaryons progressively accumulated in the *Drosophila* nuclei (data not shown). In contrast, when protein synthesis was inhibited, no human hnRNP C protein could be detected in *Drosophila* nuclei, whereas human PABP2 was readily visible (Fig. 1C–E). Human PABP2 was first detected in *Drosophila* nuclei at 3 h after fusion and progressively accumulated thereafter (data not shown). From this we conclude that during the course of the experiment human PABP2 molecules migrated from the HeLa nucleus to the cytoplasm of the heterokaryon and from there they were rapidly imported into the *Drosophila* nucleus. Thus, PABP2 shuttles between nucleus and cytoplasm, as recently reported by others (Chen et al., 1999).

Nuclear import of PABP2 is carrier mediated

PABP2 is a small protein (~33 kDa) that may cross the diffusion channel of nuclear pore complexes. A possible explanation for the observed nucleocytoplasmic shuttling could therefore be that PABP2 diffuses bidirectionally through the pores but is predominantly retained in the nucleus as it binds to the growing poly(A) tails. To address this question, we analyzed the nucleocytoplasmic distribution of PABP2 fused to nonnuclear proteins. As reporter proteins we used GFP (~27 kDa) and a form of firefly luciferase (~60 kDa) containing a Leu → Val mutation that disrupts its peroxisomal targeting signal (Gould et al., 1989). As expected for relative small proteins with no specific retention in any subcellular compartment, both GFP and luciferase appear distributed throughout the cytoplasm and the nucleus (Fig. 2A,C). In contrast, the chimeras GFP-PABP2 and luciferase-PABP2 are exclusively detected in the nucleus, excluding nucleoli (Fig. 2B,D). Moreover, both chimeras have a speckled distribution pattern in the nucleoplasm similar to endogenous PABP2 (cf. Fig. 1A). Because the fusion protein luciferase-PABP2 is too large (~90 kDa) to enter the diffusion channel of nuclear pores, we conclude that import of PABP2 into the nucleus involves a carrier-mediated mechanism.

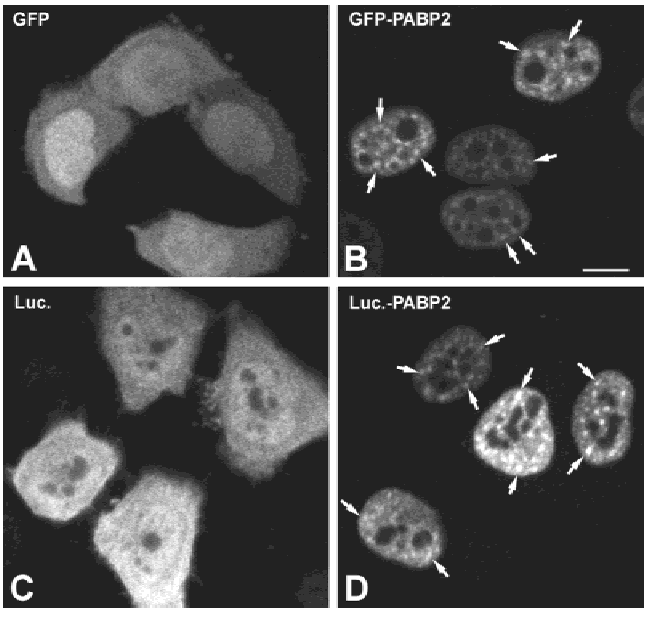


FIGURE 2. PABP2 targets heterologous proteins to the nucleus. HeLa cells were transfected with either GFP (**A**), GFP-PABP2 (**B**), luciferase (**C**), or luciferase-PABP2 (**D**). Approximately 20 h after transfection, cells expressing GFP constructs were fixed in formaldehyde and directly observed with the fluorescence microscope. Cells expressing luciferase constructs were fixed in formaldehyde, permeabilized with Triton X-100, and stained for immunofluorescence with anti-luciferase antibodies. Both PABP2 chimeras are exclusively detected in the nucleoplasm, with higher concentration in speckles (**B**,**D**, arrows). Bar = 10 μm.

A key feature of carrier-mediated nuclear transport is the recognition of specific signals present in the substrate proteins. We therefore asked which domains of PABP2 are important for its nuclear import. To investigate a potential role of the RNA-binding domain in nuclear import, we analyzed the nucleocytoplasmic distribution of a mutant form of PABP2 (dmPABP2), which contains a double-point mutation in the RNA-binding domain (phenylalanine to alanine at position 215 and tyrosine to alanine at position 175). In vitro, this mutant binds to poly(A) approximately 20-fold less than wild-type PABP2 (Nemeth, 1998). Both GFP-dmPABP2 and luciferase-dmPABP2 chimeras are exclusively detected in the nucleus (Fig. 3A and data not shown). However, in contrast with wild-type chimeras, the dmPABP2 fusion proteins are uniformly distributed in the nucleoplasm, with no concentration in speckles. Since poly(A)-enriched speckles persist in these cells, the lack of accumulation of dmPABP2 in these structures most likely reflects failure of the mutant protein to interact with poly(A) RNA (to be described in detail elsewhere). Thus, nuclear targeting of PABP2 appears independent of RNA binding.

PABP2 contains two additional structural motifs, an acidic N-terminal domain containing basic patches and an arginine-rich C-terminal domain. We therefore analyzed the localization pattern of both N- and C-terminal deletion mutants. ΔNPABP2 contains a deletion of amino acids 1–160, ΔCPABP2 contains a deletion of amino acids 257–306 (Smith et al., 1999), and CPABP2 contains only the C-terminal domain (amino acids 256–306). The fusion GFP-ΔNPABP2 is exclusively localized within the nucleus (Fig. 3B), whereas GFP-ΔCPABP2 distributes evenly between the cytoplasm and the nucleus (Fig. 3C). The fusion GFP-CPABP2 is exclusively detected in the nucleus (Fig. 3D), implying that the nuclear import signal of PABP2 is present in the C-terminal domain.

PABP2 binds transportin

The N-terminal domain of PABP2 contains a cluster of basic residues reminiscent of a classical NLS (Fig. 3, bottom diagram). However, deletion of this sequence does not affect nuclear targeting (Fig. 3B), suggesting that either PABP2 does not interact directly with transport factors or that it uses a carrier distinct from importin-α/β.

To identify a putative nuclear import receptor for PABP2, a total HeLa cell extract was subjected to binding to immobilized recombinant PABP2. As RanGTP causes the dissociation of an import substrate/import receptor complex (Rexach & Blobel, 1995; Görlich et al., 1996; Izaurralde et al., 1997b; Siomi et al., 1997), the binding of Hela cell proteins to immobilized PABP2 was compared in the absence and presence of RanGTP (Fig. 4). Among the proteins retained on the PABP2

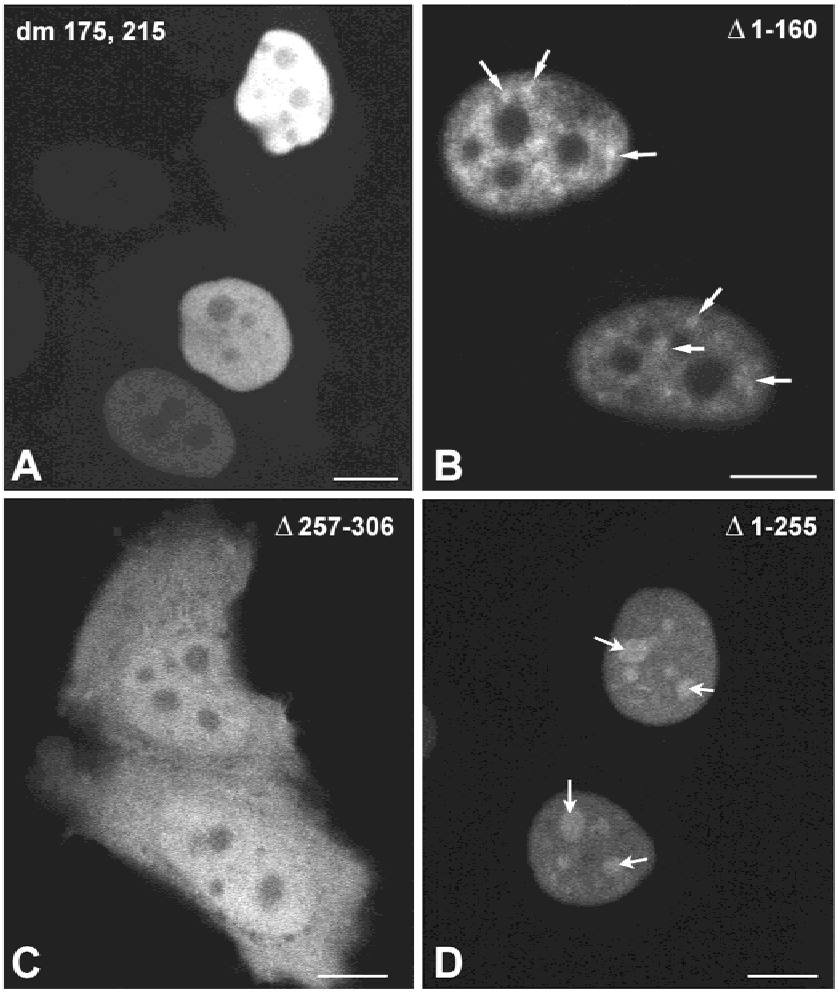
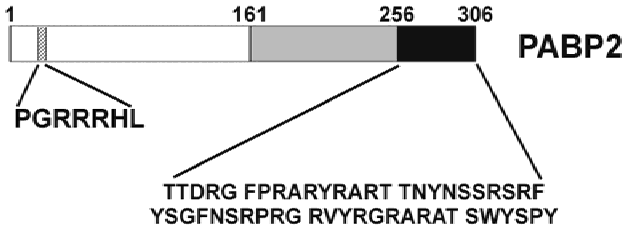


FIGURE 3. Targeting of PABP2 to the nucleus requires the C-terminal domain. The overall organization of PABP2 is schematically illustrated (bottom diagram). The protein consists of an acidic N-terminal domain (amino acids 1–160), followed by an RNA-binding domain (amino acids 161–256) and an arginine-rich C-terminal domain. The N-terminal domain contains a patch starting with P followed by three basic amino acids (R and H), which resembles a classical type of NLS. The amino acid sequence of the C-terminal domain is also indicated. The following PABP2 constructs were fused to GFP and transfected into HeLa cells: double-point mutant in the RNA-binding domain (**A**), N-terminal deletion (**B**), C-terminal deletion (**C**), and C-terminal domain (**D**). After 18–20 h, the cells were fixed and directly observed with the fluorescence microscope. Note that the chimera containing the N-terminal deletion is exclusively detected in the nucleoplasm with higher concentration in speckles (**B**, arrows), whereas the C-terminal fusion protein is concentrated in nucleoli (**D**, arrows), but not in speckles. Bar = 10 μm.



column was a prominent band of ~90 kDa that showed a RanGTP-sensitive mode of binding. Of the known nuclear transport receptors importin ß and transportin fit this molecular weight. Immunoblotting with specific antibodies to transportin and importin β demonstrated that transportin but not importin β had been specifically enriched on the PABP2 column in the absence of RanGTP. This binding of transportin to PABP2 was confirmed using recombinant transportin, which bound specifically and directly to PABP2 from a total *Escherichia coli* lysate (Fig. 4, right panel).

In conclusion, the specific and direct binding of transportin to PABP2 suggests that transportin is the nuclear import receptor for PABP2.

Nuclear export of PABP2 is carrier mediated

The results obtained with interspecies heterokaryons reveal that PABP2 molecules are transiently exported from the nucleus to the cytoplasm. Given the small size of PABP2, this could be the result of passive leakage through the pores. To investigate whether PABP2 is exported by diffusion or requires an energy-driven mechanism, we performed a temperature-shift assay, as described by Michael et al. (1995). The rational for the assay is that at 4 °C both receptor-mediated nuclear import and export are blocked, whereas diffusion is unaffected. As a positive control we fused GFP to a classical NLS (the SV40 large T antigen NLS) and ex-

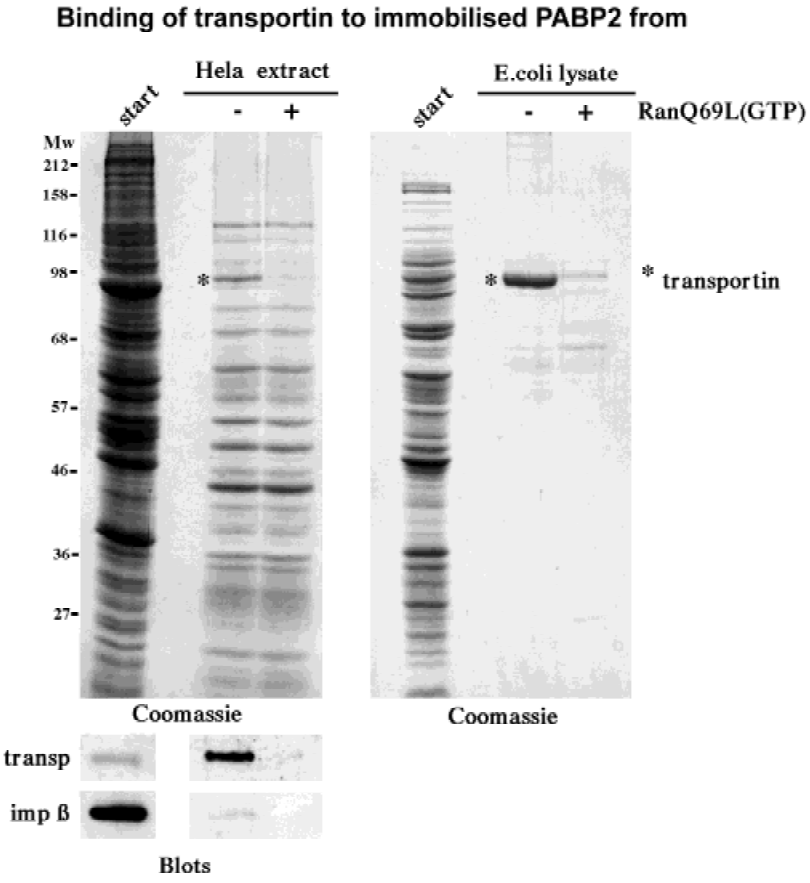


FIGURE 4. PABP2 binds transportin. A HeLa cell extract (left panels) was subjected to binding to immobilized PABP2 in the absence or presence of 2 μM RanQ69L(GTP). RanQ69L is a GTPase-deficient Ran mutant that is insensitive to cytoplasmic RanGAP1 and therefore remains in the GTP-bound form (Bischoff et al., 1994). After extensive washing, bound proteins were eluted with 1 M $MgCl_2$, precipitated and analyzed by SDS-PAGE. The Coomassie stain shows a prominent band of around 90 kDa that disappears in the presence of RanGTP (*). Western blotting confirmed that transportin but not importin β had been specifically enriched from the HeLa extract on the PABP2 column in the absence of RanGTP. Note that recombinant transportin from a total *E. coli* lysate bound specifically and directly to immobilized PABP2 (right panel). The load in the bound fractions corresponds to 50 times the starting material in the Coomassie stains and 25 times the starting material in the Western blots.

pressed it in HeLa cells (Fig. 5A,C). As GFP is a small protein (~27 kDa), it may diffuse across the pores, but due to the presence of an NLS, it is actively imported. At physiological temperature in the presence of a protein synthesis inhibitor, GFP-NLS diffuses out of the nucleus. However, because it is rapidly reimported, the protein is exclusively detected in the nucleus (Fig. 5A). In contrast, at low temperature in the absence of protein synthesis, GFP-NLS continues to leak out of the nucleus and, as NLS-mediated import is blocked, the protein progressively accumulates in the cytoplasm (Fig. 5C).

Contrasting with the results obtained with GFP-NLS, the chimera GFP-PABP2 is exclusively localized in the nucleus at both 37 °C (Fig. 5B) and 4 °C (Fig. 5D). Taking into account that GFP-PABP2 shuttles between nucleus and cytoplasm in heterokaryon assays (data not shown), we may conclude that export of PABP2 is temperature sensitive. This implies that transport of PABP2 to the cytoplasm involves a carrier-mediated pathway. Consistent with this view, a fusion protein, luciferase-PABP2 (which is too large to diffuse through the pores), retains the ability to shuttle in a heterokaryon assay (Fig. 6A,B).

Additionally, PABP2 was fused to the trypsin-resistant core of nucleoplasmin (NPc; Laskey & Dingwall, 1993). As NPc is normally retained in the nucleus (Laskey & Dingwall, 1993; Michael et al., 1995), we asked whether PABP2 can promote its export to the cytoplasm. Using the interspecies heterokaryon assay we first confirmed that a fusion protein, GFP-NPc-NLS, is not exported from the nucleus (Fig. 6C). In contrast, a fusion of this chimera with PABP2 does shuttle (Fig. 6D), demonstrating that PABP2 can direct nuclear export of a protein that is normally confined to the nucleus.

Nuclear export of PABP2 is independent of mRNA traffic

One possible explanation for the results described above is that PABP2 piggybacks on poly(A) RNAs in transit to the cytoplasm. Alternatively, export of PABP2 is the active process and the RNAs are the passive cargo. An experimental approach to distinguish these possibilities consists of determining whether export of the protein requires RNA synthesis (see Stutz & Rosbash, 1998). The rationale for the experiment is the following. If PABP2 is a passive cargo of mRNA, then treatment of cells with actinomycin D should prevent shuttling, because in the absence of ongoing transcription there is no traffic of mRNA to the cytoplasm. In contrast, if export of PABP2 is the active process it should continue in the absence of RNA synthesis, as previously

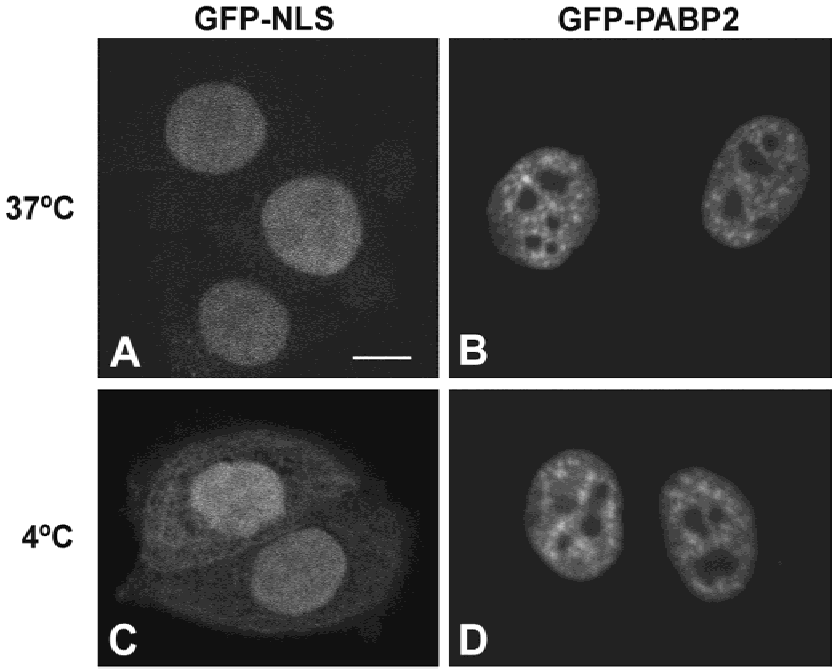


FIGURE 5. Temperature-sensitive nuclear export of PABP2. HeLa cells were transfected with GFP-NLS (**A**,**C**) or GFP-PABP2 (**B**,**D**). After 18–20 h, the cells were treated with a protein synthesis inhibitor (20 μg/mL emetine) for 3 h and then incubated in media containing emetine for another 3 h at 37 °C (**A**,**B**) or 4 °C (**C**,**D**). At 37 °C both proteins are exclusively detected in the nucleus due to active import. At 4 °C, GFP-NLS is detected in the cytoplasm because it passively diffuses out of the nucleus whereas active nuclear import is blocked; in contrast, GFP-PABP2 remains restricted to the nucleus, indicating that it does not leak to the cytoplasm by a passive event. Bar = 10 μm.

demonstrated for hnRNP A1 proteins (Piñol-Roma & Dreyfuss, 1992).

HeLa cells were incubated with actinomycin D for 3 h before fusion to *Drosophila* SL2 cells. As shown in Figure 7A–C, export of PABP2 continues under these conditions. A similar result is observed when HeLa cells expressing either GFP-PABP2 or luciferase-PABP2 were incubated with actinomycin D for 3 h before fusion to mouse 3T3 cells (Fig. 7D–E,G–H). Incubation of actinomycin D-treated cells at 4 °C confirms that neither GFP-PABP2 nor luciferase-PABP2 chimeras diffuse passively to the cytoplasm (Fig. 7F,I). Thus, PABP2 is exported to the cytoplasm by a carrier-mediated pathway that is independent of mRNA traffic.

DISCUSSION

Export of mRNA from the nucleus is an energy-dependent process that relies on recognition of the RNA substrate by specific transport receptors. Because mRNAs in the nucleus associate tightly with several proteins forming ribonucleoprotein particles (hnRNPs or mRNPs), it is generally assumed that transport is mediated by interaction of the receptors

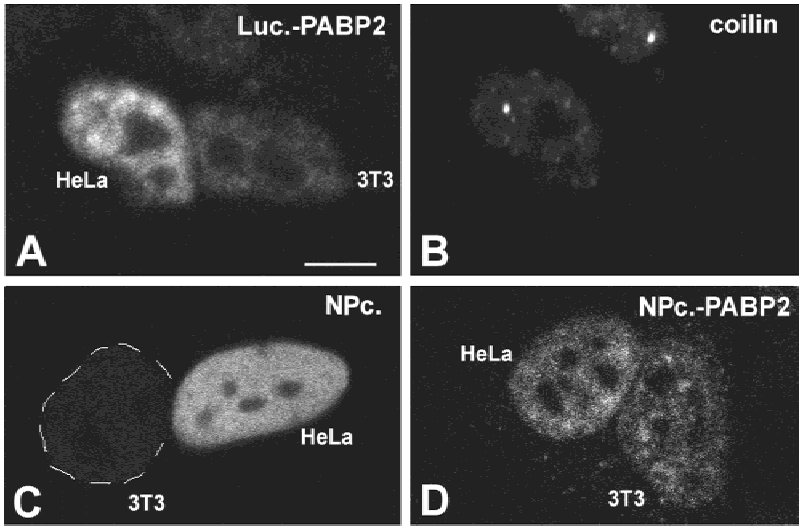


FIGURE 6. PABP2 export involves a receptor-mediated pathway. HeLa cells were transfected with the following fusion proteins: luciferase-PABP2 (**A**,**B**), GFP-nucleoplasmin core (NPc)-NLS (**C**) or GFP-nucleoplasmin core (NPc)-NLS-PABP2 (**D**). Heterokaryons were formed with mouse NIH 3T3 cells and incubated for 3–6 h in the presence of 20 μg/mL emetine. Immunostaining with anti-coilin antibody was used as a control because this protein is retained in the nucleus (**B**). Note that both PABP2 chimeras shuttle from the HeLa to the 3T3 nuclei. Bar = 10 μm.

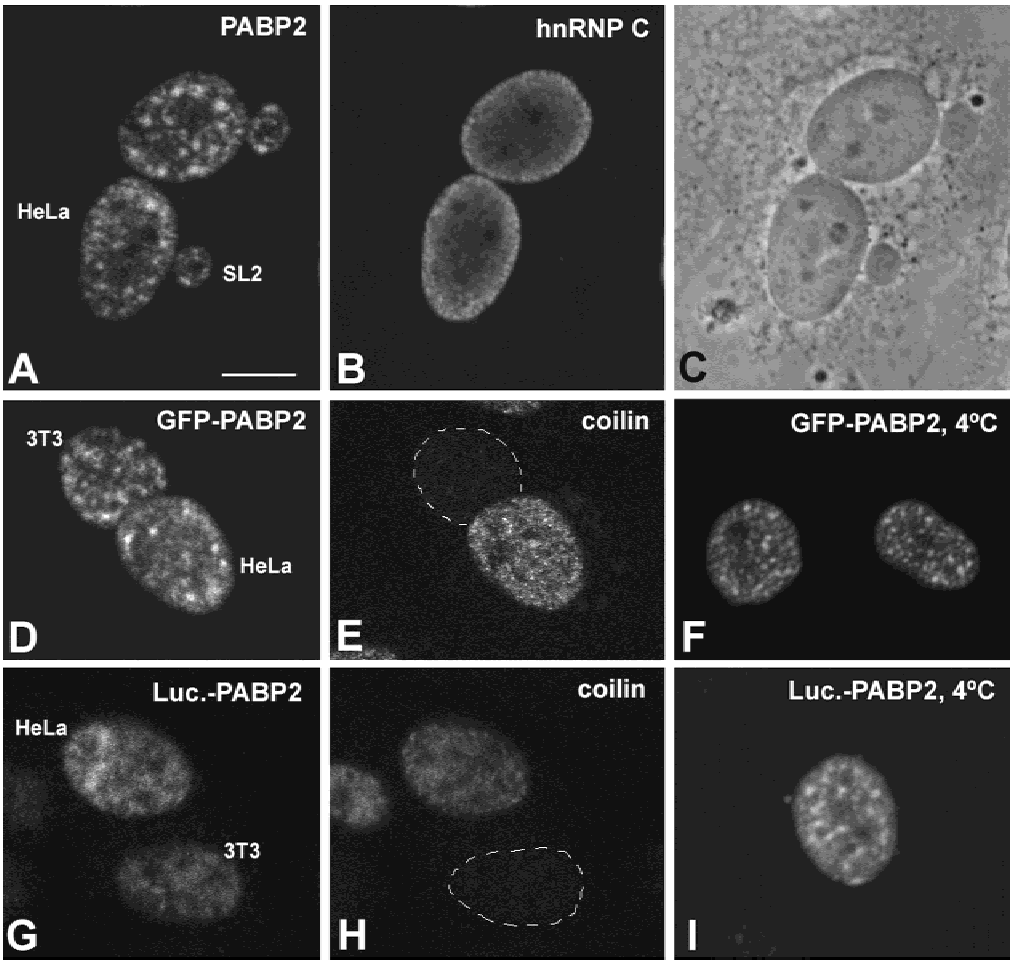


FIGURE 7. PABP2 export is independent from ongoing transcription. **A**--**C**: HeLa cells were cocultured with *Drosophila* SL2 cells and treated with inhibitors of protein synthesis and transcription (20 μg/mL emetine and 5 μg/mL actinomycin D) for 3 h. After PEG-induced fusion, the cells were incubated in medium containing both inhibitors for another 3–6 h. The resulting heterokaryons were observed by phase contrast microscopy (**C**) and double labeled with anti-PABP2 and hnRNP C antibodies (**A**,**B**). HeLa cells expressing GFP-PABP2 or luciferase-PABP2 were similarly treated with emetine and actinomycin D and fused to mouse 3T3 cells. **D**, **E**, **G**, and **H** show that both PABP2 chimeras shuttle from the Hela to the 3T3 nuclei. To exclude that nuclear export of PABP2 is due to diffusion, cells expressing these chimeras were treated with emetine and actinomycin D for 3 h and then incubated at 4 °C for another 3 h (**F**,**I**). Bar = 10 μm.

with specific nuclear export signals present in the RNA-binding proteins (Lee & Silver, 1997; Nakielny et al., 1997; Stutz & Rosbash, 1998). This model is supported by the finding that a subset of hnRNP proteins shuttle between the nucleus and the cytoplasm and contain a transferable nuclear export signal. In addition to hnRNP proteins, the poly(A) tails of all mRNAs in the nucleus of mammalian cells are coated with molecules of poly(A)-binding protein II (Kühn & Wahle, 1997). The data reported here demonstrate that similarly to the shuttling hnRNP proteins, PABP2 is exported to the cytoplasm by a carrier-mediated mechanism that is independent of mRNA traffic. Thus, PABP2 may also participate in the process of mRNA transport.

The overall organization of PABP2, with an acidic N-terminal domain containing basic patches, followed by an RNA-binding domain and an arginine-rich C-terminal domain, is reminiscent of the nucleolar protein nucleolin (Nemeth et al., 1995), which also shuttles between the nucleus and the cytoplasm (Borer et al., 1989). In this regard it is noteworthy that the C-terminal domain of PABP2 expressed as a chimera with GFP is predominantly localized in the nucleolus, whereas all other forms of the protein are excluded from this compartment (cf. Fig. 3).

All macromolecules in transit between nucleus and cytoplasm are routed through the nuclear pore complexes, which form aqueous channels penetrating and fusing the double bilayer membrane of the nuclear envelope. Nuclear pore complexes contain a diffusion channel 9 nm in diameter that allows passive movement of small molecules in and out of the nucleus, whereas globular molecules greater then 60–70 kDa

fail to diffuse across the pores at a significant rate. However, macromolecular complexes as large as 25–50 MDa can be translocated through the pores by active or facilitated transport mechanisms. In response to specific signals, the nuclear pore complex undergoes a change in conformation and expands the channel to a maximum of approximately 25 nm in diameter (for recent reviews, see Nigg, 1997; Ohno et al., 1998).

The predicted molecular weight of PABP2 is ~33 kDa (Nemeth et al., 1995) and therefore, if the protein is present in the cell as free monomers, it is expected to passively diffuse through the pores and therefore equilibrate between the nucleus and the cytoplasm. However, our finding that PABP2 targets to the nucleus a fusion protein (luciferase-PABP2, predicted molecular weight ~90 kDa) that is too large to cross the pores by simple diffusion provides evidence for involvement of a carrier-mediated mechanism. This is further supported by the result that PABP2 binds directly and specifically to the nuclear import receptor transportin.

Until a few years ago, nuclear import studies were centered on proteins carrying either a mono- or a bipartite NLS being recognized by a heterodimeric carrier named importin α/β. However, recent work led to the discovery of a second pathway for protein import mediated by an importin-β-related protein, termed transportin in mammals (Pollard et al., 1996) and Kap104p in yeast (Aitchison et al., 1996). In mammals transportin mediates import of a subset of hnRNP proteins, the best characterized of which is hnRNP A1 (Pollard et al., 1996; Siomi et al., 1997). The signal within hnRNP A1 that mediates its nuclear import is a stretch of 38 C-terminal amino acids termed the M9 domain (Siomi & Dreyfuss, 1995). Because hnRNP proteins are very abundant (10^6–10^7 copies per cell), it has been speculated that transportin has evolved as a specialized carrier for these high abundance substrates, contrasting with the importin-α/β carrier that recognizes a broad range of cargoes but with most members present at relatively low abundance (Nigg, 1997, Görlich, 1997). The data presented here demonstrate that PABP2 binds directly to transportin, thus providing evidence that PABP2 is a novel substrate for this carrier. Importantly, PABP2 dissociates from transportin in the presence of RanGTP, suggesting that, in vivo, the high concentration of RanGTP in the nucleus triggers release of the cargo (PABP2) into the nucleoplasm, as previously demonstrated for the importin-α/β-mediated import pathway (Görlich, 1997; Izaurralde et al., 1997b; Siomi et al., 1997). The data also suggest that the NLS of PABP2 is contained in its C-terminal domain. However, the C-terminal sequence of PABP2 (Fig. 3, bottom diagram) shows no apparent homology with the M9 signal, raising the possibility that it represents a novel type of NLS.

An interesting feature of the C-terminus of PABP2 is that it is very rich in arginine residues, almost all of which are dimethylated (Smith et al., 1999). N^G,N^G-dimethylarginine is also a common modified amino acid in hnRNP proteins (Beyer et al., 1977; Boffa et al., 1977), and more recently, arginine methylation was shown to be important for the nuclear export of shuttling hnRNP proteins in yeast (Shen et al., 1998). Therefore, an important question to be addressed in future studies is whether dimethylated arginines in the C-terminal domain of PABP2 play a role in transport of the protein in and out of the nucleus.

In conclusion, the results presented here indicate that PABP2 is exported to the cytoplasm by a facilitated transport pathway that acts independently from RNA synthesis, thus suggesting that this protein may play a role in mRNA transport similarly to shuttling hnRNPs (Pollard et al., 1996; Izaurralde et al., 1997a).

MATERIALS AND METHODS

Cell culture, immunofluorescence, and *in situ* hybridization

HeLa and NIH 3T3 cells were cultured as monolayers in Modified Eagle's medium (MEM) and Dulbecco's Modified Eagle's medium (DME), respectively. Drosophila SL2 cells were grown in suspension in Schneider's Medium. All media were supplemented with 10% fetal calf serum (FCS; Gibco).

Cells grown on 10 × 10 mm coverslips were briefly rinsed in phosphate buffered saline (PBS) and fixed in 3.7% formaldehyde (freshly prepared from paraformaldehyde) diluted in either PBS or HPEM buffer (30 mM HEPES, 65 mM PIPES, 10 mM EGTA, 2 mM $MgCl_2$, pH 6.9) for 10 min at room temperature. The cells were then permeabilized with 0.5% Triton X-100 in PBS (or HPEM buffer) for 10 min at room temperature.

For immunofluorescence, the cells were rinsed in PBS containing 0.05% Tween 20 (PBS-Tw), incubated for 1 h with primary antibodies diluted in PBS, washed in PBS-Tw, and incubated with appropriate secondary antibodies for 30 min. After washing in PBS-Tw, the samples were mounted in VectaShield (Vector Laboratories, UK). Secondary antibodies conjugated to FITC or Texas red were obtained from Jackson ImmunoResearch Laboratories, USA.

Endogenous PABP2 was visualized using a polyclonal serum previously described (Krause et al., 1994). Affinity-purified antibodies were obtained using recombinant PABP2 expressed in *E. coli* from plasmid pGM10-PABP2 (Smith et al., 1999). Additionally, the following antibodies were used: monoclonal antibody 4F4 directed against hnRNP C protein (Choi & Dreyfuss, 1984); rabbit polyclonal antibodies directed against luciferase (Promega); and monoclonal antibody delta directed against human p80 coilin (Almeida et al., 1998).

In situ hybridization to detect poly(A) RNA was performed as described previously (Gama-Carvalho et al., 1997).

Construction and expression of fusion proteins

The cDNA encoding bovine PABP2 was subcloned from the pGM10-PABP2 prokaryotic expression vector (Smith et al.,

1999) into the pEGFP-C1 protein fusion vector (Clontech). pGM10-PABP2 was cut with *Nde*I and the recessed 3′ ends were filled in. The linear plasmid was then cut with *Bam*HI and the *Nde*I(filled in)-*Bam*HI fragment encoding the cDNA of PABP2 was purified. The pEGFP-C1 was cut with *Sma*I and *Bam*HI, and ligated to the cDNA fragment. The same strategy was used to subclone the cDNAs for PABP2 mutants.

To construct the pEGFP-NLS and pEGFP-NLS-PABP2 expression vectors, two complementary oligonucleotides with the sequences 5′-A GCT TGG ATC CCT AAG AAG AAG CGA AAG GTT TCT GCA-3′ (5′ → 3′) and 5′-GA AAC CTT TCG CTT CTT CTT AGG GAT CCA-3′ (3′ → 5′) were synthesized (Gibco-BRL). The annealed oligonucleotide product was inserted between the EGFP gene and the cDNA of PABP2 using the *Hin*dIII and *Pst*I sites of the pEGFP-PABP2 vectors. This introduces the sequence encoding the NLS of the large T antigen from the SV40 virus into pEGFP and pEGFP-PABP2 vectors. The oligonucleotide also provides a new restriction site for *Bam*HI that was used to confirm insertion. A similar approach was used to construct the pEGFP-NLS vector.

To fuse PABP2 to luciferase, we have made use of plasmid pBluescript KS+LL/V. First, this plasmid was cut with *Hin*dIII and *Pst*I, and the fragment encoding the luciferase gene was purified. This was subcloned into the *Hin*dIII and *Pst*I sites of pSE280 (Invitrogen). This plasmid was then cut with *Eco*NI and the recessed 3′ ends generated were filled in. Subsequently the linear plasmid was cut with *Bgl*II. The product containing the luciferase gene deleted in 31 nt at the 3′ end was purified and ligated to the cDNAs of PABP2 isolated as described above. Finally, the pSE280-luciferase-PABP2 vector was cut with *Hin*dIII and *Sal*I, and the fragment containing the luciferase-PABP2 fusion gene was then purified and subcloned into the *Hin*dIII and *Sal*I sites of pCMV5 (Andersson et al., 1989), thus generating the pCMV5-Luciferase-PABP2 vector. To produce the pCMV5-luciferase plasmid, the *Hin*dIII-*Pst*I fragment encoding the luciferase gene was subcloned into the *Hin*dIII and *Pst*I sites of pCMV5.

The pEGFP-NPc-NLS and pEGFP-NPc-NLS-PABP2 expression vectors were constructed from plasmid pcDNA3-myc-NPc, which contains the sequence that encodes amino acids 2–150 of the trypsin-resistant NPc (Michael et al., 1995). This plasmid was cut with *Eco*RI and the recessed 3′ ends were filled in. The plasmid was then cut with *Bam*HI and the NPc-encoding fragment was purified. The pEGFP-NLS and pEGFP-NLS-PABP2 vectors were cut with *Hin*dIII, and the recessed 3′ ends filled in. The linear plasmids were cut with *Bgl*II and then ligated with the NPc-coding fragment.

Final DNA constructs were purified using the Qiagen plasmid midi kit, and transfection of HeLa cells was performed using either the calcium phosphate method (Sambrook et al., 1989), Lipofectin (Gibco, BRL) or FuGene 6 (Boehringer Mannheim), according to the manufacturer's instructions.

Heterokaryon assay

For HeLa × SL2 heterokaryons, HeLa cells were grown to subconfluent density on glass coverslips for 1 day. To induce adhesion, the SL2 cells were washed and seeded onto the HeLa coverslips in serum-free MEM for 15 min at 29 °C in the presence of 5% CO_2. Then the medium was replaced by MEM supplemented with 10% FCS, and the cells were incubated for 3 h at 29 °C in an atmosphere of 5% CO_2, in either presence or absence of emetine (20 μg/mL). To induce cell fusion, the coverslips were rinsed in PBS and placed on a drop of polyethylene-glycol (PEG 1500; Boehringer Mannheim) for 2 min. The coverslips were then washed in PBS and further incubated in culture medium for periods of 1, 2, 3, and 6 h. For HeLa × 3T3 heterokaryons, 3T3 cells were seeded onto the HeLa coverslips (at subconfluent density) and allowed to adhere for 3 h in DME supplemented with 10% FCS. After cell fusion, the heterokaryons were incubated for 3–6 h.

Microscopy

Fluorescently labeled samples were analyzed using the laser scanning microscope Zeiss LSM410 equipped with an Argon Ion laser (488 nm) to excite FITC fluorescence and a helium-neon laser (543 nm) to excite Texas Red fluorescence. For double-labeling experiments, images from the same focal plane were sequentially recorded in different channels and superimposed. To obtain a precise alignment of superimposed images, the equipment was calibrated using multicolor fluorescent beads (Molecular Probes, Eugene, Oregon), and a dual-band filter that allows simultaneous visualization of red and green fluorescence.

Cloning and expression of recombinant zzPABP2

A construct for the expression of a zz-tagged PABP2 was obtained by cloning a *Nco*I/*Bam*HI fragment from pGM10-PABP2 into the zz-60 vector. This vector is a derivative of pQE60 with two z tags (IgG-binding domain of protein A). The resulting fusion contains two N-terminal z-tags followed by a His-tag and PABP2. zzPABP2 was expressed in *E. coli* XL 1 at 25 °C. Disruption of the cells and purification on nickel-NTA agarose was in 2× PBS.

Binding assays

The preparation of the HeLa extract was as described (Kutay et al. 1997). The expression and purification of recombinant human transportin and RanQ69L have also been described (Izaurralde et al., 1997b).

Recombinant zzPABP2 was immobilized on IgG-Sepharose 4B at a concentration of 1 mg/mL of resin. All binding experiments were performed in 50 mM HEPES, pH 7.6, 150 mM NaCl, 75 mM potassium acetate and 5 mM magnesium acetate. For each sample, 20 μL of affinity matrix were incubated with either 500 μL of HeLa extract or 200 μL of transportin lysate in a total volume of 1.5 mL for 4 h at 4 °C. Where indicated RanQ69L was added to 2 μM. After binding, beads were washed four times with 50 mM HEPES, pH 7.6, 200 mM NaCl, 25 mM potassium acetate, and 5 mM magnesium acetate. Elution was with 50 mM Tris-HCl, pH 7.5, 1 M $MgCl_2$. Samples were precipitated with 90% isopropanol and dissolved in SDS sample buffer.

ACKNOWLEDGMENTS

We wish to acknowledge Professor David Ferreira for encouragement, Dr. Dirk Görlich for support and stimulating discussions, and Dr. Domingos Henrique for helpful advice

on cloning experiments. We are also grateful to Dr. A. Nemeth for generously providing PABP2 mutants; Dr. D. Görlich and Dr. G. Dreyfuss for antibodies directed against importin β, transportin, and hnRNP proteins; Dr. O. Bensaude for luciferase plasmid; and Dr. G. Dreyfuss for pcDNA-myc-NPc plasmid. This study was supported by grants from Fundaçãao para a Ciência e Tecnologia/PRAXIS XXI and from the European Union (A.C. and M.C.-F.); Deutsche Forschungsgemeinschaft (U.K. and E.W.) and Fonds der Chemischen Industrie (E.W.). A.C. is a fellow from the Gulbenkian Ph.D. Program.

Received August 20, 1999; returned for revision October 10, 1999; revised manuscript received October 19, 1999

REFERENCES

Adesnik M, Salditt W, Thomas W, Darnell JE. 1972. Evidence that all messenger RNA molecules (except histone messenger RNA) contain poly(A) sequences and that the poly(A) has a nuclear function. *J Mol Biol 71*:21–30.

Afonina E, Neumann M, Pavlakis GN. 1997. Preferential binding of poly(A)-binding protein 1 to an inhibitory RNA element in the Human Immunodeficiency Virus type 1 *gag* mRNA. *J Biol Chem 272*:2307–2311.

Afonina E, Stauber R, Pavlakis GN. 1998. The human poly(A)-binding protein 1 shuttles between the nucleus and the cytoplasm. *J Biol Chem 273*:13015–13021.

Aitchison JD, Bolbel G, Rout MP. 1996. Kap104p: A karyopherin involved in the nuclear transport of messenger RNA binding proteins. *Science 274*:624–627.

Almeida F, Saffrich R, Ansorge W, Carmo-Fonseca M. 1998. Microinjection of anti-coilin antibodies affects the structure of coiled bodies. *J Cell Biol 142*:899–912.

Amrani N, Minet M, Le Gouar M, Lacroute F, Wyers F. 1997. Yeast Pab1 interacts with Rna15 and participates in the control of the poly(A) tail length in vitro. *Mol Cell Biol 17*:3694–3701.

Andersson S, Davis DC, Dahlback H, Jornvall H, Russel PW. 1989. Cloning, structure and expression of the mitochondrial cytochrome P-450 sterol 26-hydrolase, a bile acid biosynthetic enzyme. *J Biol Chem 264*:8222–8229.

Beelman CA, Parker R. 1995. Degradation of mRNA in eukaryotes. *Cell 81*:179–183.

Bernstein P, Peltz SW, Ross J. 1989. The poly(A)-binding protein complex is a major determinant of mRNA stability in vitro. *Mol Cell Biol 9*:659–670.

Beyer AL, Christensen AO, Walker BW, LeStourgeon WM. 1977. Identification and characterization of the packaging proteins of core 40S hnRNP particles. *Cell 11*:127–138.

Bienroth S, Keller W, Wahle E. 1993. Assembly of a processive messenger RNA polyadenylation complex. *EMBO J 12*:585–594.

Bischoff FR, Klebe C, Kretschmer J, Wittinghofer A, Ponstingl H. 1994. RanGAP1 induces GTPase activity of nuclear ras-related Ran. *Proc Natl Acad Sci USA 91*:2587–2591.

Boeck R, Lapeyre B, Brown CE, Sachs AB. 1998. Capped mRNA degradation intermediates accumulate in the yeast spb8-2 mutant. *Mol Cell Biol 18*:5062–5072.

Boffa LC, Karn J, Vidali G, Allfrey VG. 1977. Distribution of N^G,N^G-dimethylarginine in nuclear protein fractions. *Biochem Biophys Res Commun 74*:969–976.

Borer RA, Lehner CF, Eppenberger HM, Nigg EA. 1989. Major nucleolar protein shuttles between nucleus and cytoplasm. *Cell 56*:379–390.

Brown CE, Sachs AB. 1998. Poly(A) tail length control in *Saccharomyces cerevisiae* occurs by message-specific deadenylation. *Mol Cell Biol 18*:6548–6559.

Caponigro G, Parker R. 1995. Multiple functions for the poly(A)-binding protein in mRNA decapping and deadenylation in yeast. *Genes & Dev 9*:2421–2432.

Carter KC, Taneja KL, Lawrence JB. 1991. Discrete nuclear domains and their relationship to the functional organization of the nucleus. *J Cell Biol 115*:1191–1202.

Chen Z, Li Y, Krug R. 1999. Influenza A virus NS1 protein targets poly(A)-binding protein II of the cellular 3′-end processing machinery. *EMBO J 18*:2273–2283.

Choi YD, Dreyfuss G. 1984. Monoclonal antibody characterization of the C proteins of heterogeneous nuclear ribonucleoprotein complexes in vertebrate cells. *J Cell Biol 99*:1997–2204.

Colgan DF, Manley J. 1997. Mechanism and regulation of mRNA polyadenylation. *Genes & Dev 11*:2755–2766.

Coller JM, Gray NK, Wickens MP. 1998. mRNA stabilization by poly(A) binding protein is independent of poly(A) and requires translation. *Genes & Dev 12*:3226–3235.

Craig AWB, Haghighat A, Yu ATK, Sonenberg N. 1998. Interaction of polyadenylate-binding protein with the eIF4G homologue PAIP enhances translation. *Nature 392*:520–523.

Ford LP, Bagga PS, Wilusz J. 1997. The poly(A) tail inhibits the assembly of a 3′-to-5′ exonuclease in an in vitro RNA stability system. *Mol Cell Biol 17*:398–406.

Gama-Carvalho M, Krauss RD, Chiang L, Valcárcel J, Green MR, Carmo-Fonseca M. 1997. Targeting of $U2AF^{65}$ to sites of active splicing in the nucleus. *J Cell Biol 137*:975–987.

Görlach M, Burd CG, Dreyfuss G. 1994. The mRNA poly(A)-binding protein: Localization, abundance, and RNA-binding specificity. *Exp Cell Res 211*:400–407.

Görlich D. 1997. Nuclear protein import. *Curr Opin Cell Biol 9*:412–419.

Görlich D, Panté N, Kutay U, Aebi U, Bischoff FR. 1996. Identification of different roles for RanGDP and RanGTP in nuclear protein import. *EMBO J 15*:5584–5594.

Gould SJ, Keller GA, Hosken N, Wilkinson J, Subramani S. 1989. A conserved tripeptide sorts proteins to peroxisomes. *J Cell Biol 108*:1657–1664.

Izaurralde E, Jarmolowski A, Beisel C, Mattaj IW, Dreyfuss G, Fischer U. 1997a. A role for the hnRNPA1 M9-transport signal in mRNA nuclear export. *J Cell Biol 137*:27–35.

Izaurralde E, Kutay U, von Kobbe C, Mattaj IW, Görlich D. 1997b. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. *EMBO J 16*:6535–6547.

Keller W. 1995. No end yet to messenger RNA 3′ end processing. *Cell 81*:829–832.

Keller W, Minvielle-Sebastia L. 1997. A comparison of a mammalian and yeast pre-mRNA 3′-end processing. *Curr Opin Cell Biol 9*:329–336.

Körner CG, Wahle E. 1997. Poly(A) tail shortening by a mammalian poly(A)-specific 3′-exoribonuclease. *J Biol Chem 272*:10448–10456.

Krause S, Fakan S, Weis K, Wahle E. 1994. Immunodetection of poly(A)-binding protein II in the cell nucleus. *Exp Cell Res 214*:75–82.

Kühn U, Wahle E. 1997. The mechanism of 3′ cleavage and polyadenylation of eukaryotic pre-mRNA. *Prog Nucleic Acid Res Mol Biol 57*:41–72.

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

Laskey RA, Dingwall C. 1993. Nuclear shuttling: The default pathway for nuclear proteins? *Cell 74*:585–586.

Le H, Tanguay RL, Balasta ML, Wei C, Browning KS, Metz AM, Goss DJ, Gallie DR. 1997. Translation initiation factors eIF-iso4G and eIF-4B interact with the poly(A)-binding protein and increase its RNA binding activity. *J Biol Chem 272*:16247–16255.

Lee MS, Silver P. 1997. RNA movement between the nucleus and the cytoplasm. *Curr Opin Genet Dev 7*:212–219.

Manley JL. 1995. A complex protein assembly catalyzes polyadenylation of mRNA precursors. *Curr Opin Genet Dev 5*:222–228.

Michael WM, Choi M, Dreyfuss G. 1995. A nuclear export signal in hnRNP A1: A signal-mediated, temperature-dependent nuclear export pathway. *Cell 83*:415–422.

Minvielle-Sebastia L, Preker PJ, Wiederkehr T, Strahm Y, Keller W. 1997. The major yeast poly(A)-binding protein is associated with cleavage factor IA and functions in pre-messenger RNA 3′-end formation. *Proc Natl Acad Sci USA 94*:7897–7902.

Nakielny S, Dreyfuss G. 1997. Nuclear export of proteins and RNAs. *Curr Opin Cell Biol 9*:420–429.

Nakielny S, Fischer U, Michael WM, Dreyfuss G. 1997. RNA transport. *Annu Rev Neurosci 20*:269–301.
Nemeth A. 1998. Cloning and characterisation of the domain structure of bovine poly(A) binding protein II and of the homologous protein (ROX2) from *Drosophila melanogastor*. Ph.D. Thesis, University of Gießen, Germany.
Nemeth A, Krause S, Blank D, Jenny A, Jenö P, Lustig A, Wahle E. 1995. Isolation of genomic and cDNA clones encoding poly(A) binding protein II. *Nucleic Acids Res 23*:4034–4041.
Nigg EA. 1997. Nucleocytoplasmic transport: Signals, mechanisms and regulation. *Nature 386*:779–787.
Ohno M, Fornerod M, Mattaj IW. 1998. Nucleocytoplasmic transport: The last 200 nanometers. *Cell 92*:327–336.
Piñol-Roma S, Dreyfuss G. 1992. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. *Nature 355*:730–732.
Pollard VW, Michael WM, Nakielny S, Siomi MC, Dreyfuss G. 1996. A novel receptor-mediated nuclear protein import pathway. *Cell 86*:985–994.
Rexach M, Blobel G. 1995. Protein import into nuclei: Association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell 83*:683–692.
Sachs AB, Bond MW, Kornberg RD. 1986. A single gene from yeast for both nuclear and cytoplasmic polyadenylate-binding proteins: Domain structure and expression. *Cell 45*:827–835.
Sachs AB, Davis RW. 1989. The poly(A) binding protein is required for poly(A) shortening and 60S ribosomal subunit-dependent translation initiation. *Cell 58*:857–867.
Sachs AB, Deardorff JA. 1992. Translation initiation requires the PAB-dependent poly(A) ribonuclease in yeast. *Cell 70*:961–973.
Sachs AB, Sarnow P, Hentze MW. 1997. Starting at the beginning, middle and end: Translation initiation in eukaryotes. *Cell 89*:831–838.
Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
Shen EC, Henry MF, Weiss VH, Valentini SR, Silver P, Lee MS. 1998. Arginine methylation facilitates the nuclear export of hnRNP proteins. *Genes & Dev 12*:679–691.
Siomi H, Dreyfuss G. 1995. A nuclear localization domain in the hnRNP A1 protein. *J Cell Biol 129*:551–560.
Siomi MC, Eder PS, Kataoka N, Wan L, Liu Q, Dreyfuss G. 1997. Transportin-mediated nuclear import of heterogeneous nuclear RNP proteins. *J Cell Biol 138*:1181–1192.
Smith JJ, Rücknagel KP, Schierhorn A, Tang J, Nemeth A, Linder M, Herschman HR, Wahle E. 1999. Unusual sites of arginine methylation in poly(A) binding protein II and in vitro methylation by protein arginine methyltransferases PRMT1 and PRMT3. *J Biol Chem 274*:13229–13234.
Stutz F, Rosbash M. 1998. Nuclear RNA export. *Genes & Dev 12*:3303–3319.
Tarun S, Sachs AB. 1995. A common function for mRNA 5′ and 3′ ends in translation initiation in yeast. *Genes & Dev 9*:2997–3007.
Tarun S, Sachs AB. 1996. Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. *EMBO J 15*:7168–7177.
Wahle E. 1991. A novel poly(A)-binding protein acts as a specificity factor in the second phase of messenger RNA polyadenylation. *Cell 66*:759–768.
Wahle E. 1995. Poly(A) tail length control is caused by termination of processive synthesis. *J Biol Chem 270*:2800–2808.
Wormington M, Searfoss AM, Hurney CA. 1996. Overexpression of poly(A) binding protein prevents maturation-specific deadenylation and translational inactivation in *Xenopus* oocytes. *EMBO J 15*:900–909.