

The Human Poly(A)-binding Protein 1 Shuttles between the Nucleus and the Cytoplasm*

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We have studied the intracellular localization of poly(A)-binding protein 1 (PABP1) by indirect immunofluorescence as well as by tagging with the green fluorescent protein (GFP) in living cells. We show that PABP1 is able to enter the nucleus. Accumulation of PABP1 in the nuclei was observed upon transcription inhibition, suggesting that active transcription is required for PABP1 export. The nuclear import of PABP1 is an energy-dependent process since PABP1 fails to enter the nucleus upon ATP depletion and at low temperature. Transfection of PABP1 or PABP1-GFP resulted in heterogeneity of intracellular distribution of the protein. In the low expressing cells, PABP1 was localized in the cytoplasm, whereas in the high expressors, we observed accumulation of the protein in the nucleus. Nuclear PABP1 observed either after overexpression or after transcription inhibition was found in speckles and colocalized with splicing factor SC35. The ability of PABP1 to shuttle between nucleus and cytoplasm was also shown by heterokaryon formation upon cell fusion. Deletion mutagenesis showed that the minimal part of PABP1 retaining the ability to shuttle consists of the first two RNA-binding domains. This mutant interacted with poly(A) RNA with high affinity and accumulated in the nucleus. Deletion mutants exhibiting reduced RNA binding affinity did not accumulate in the nucleus. PABP1 has been proposed to participate at various steps of mRNA utilization. Our results suggest involvement of PABP1 in nuclear events associated with the formation and transport of mRNP to the cytoplasm and identify a new trafficking pattern for RNA-binding proteins.

Eukaryotic mRNAs are organized in ribonucleoprotein complexes (1, 2). One predominant protein of these complexes is the poly(A)-binding protein 1 (PABP1)¹, which associates with the 3' poly(A) tail of mRNA (3–6). PABP1 is an essential protein in

yeast (7) and is highly conserved among eukaryotic organisms (3, 4). PABP1 is clearly a multifunctional protein, proposed to participate in 3' end formation of mRNA, translation initiation, mRNA stabilization, protection of poly(A) from nuclease activity, mRNA deadenylation, inhibition of mRNA decapping, and mRNP maturation (8, 9). PABP1 may be a key factor in mediating regulation of mRNA turnover through the inhibition of mRNA decapping by the poly(A) tail or by influencing the rate of deadenylation (10–13). Several lines of evidence argue that PABP1 plays a role in stimulating translation initiation (14–16), suggesting that the interaction of this protein with the 3' poly(A) sequence can influence events at the 5' end of mRNA. PABP1 availability for interaction with processed mRNA is very important. Recent studies have shown that PABP1 is involved in the processing of 3' end of premessenger RNA (9). These results imply that interaction of PABP1 with poly(A) tail occurs in the nucleus. Mature mRNA is exported from the nucleus accompanied by several hnRNP proteins (17–19). Presence of PABP1 on the poly(A) tails of exported mRNA could be advantageous for translation initiation.

Recently, we have shown that PABP1 can also bind internal mRNA sites and that binding to the inhibitory/instability sequences (INS1) of human immunodeficiency virus type 1 (HIV-1) may affect HIV-1 mRNA stability and utilization (20). Since our previous experiments suggested that the presence of INS1 affects not only expression but also the export of gag mRNA (21, 22), we examined the possibility that interaction with PABP1 already occurs in the nucleus. PABP1 is thought to be a cytoplasmic protein and has been detected in the cytoplasm by immunofluorescence (23).

In this report, we show that PABP1 is not a purely cytoplasmic protein, but it is also found in the nucleus. We studied the localization of PABP1 by using both indirect immunofluorescence and tagging of the PABP1 by fusion to the green fluorescent protein (GFP). We present data demonstrating that PABP1 shuttles between the nucleus and cytoplasm. The RNA-binding ability of PABP1 is important for nuclear retention. These results suggest an expanded role for PABP1 in nucleocytoplasmic trafficking and utilization of mRNP particles.

MATERIALS AND METHODS

Recombinant DNA—Plasmid pGEM1 containing the coding sequence of human PABP1 was obtained from T. Grange (Institut Jacques Monod, France) (3). The PABP1 coding sequence was inserted into the *Bss*HII-*Bam*HI sites of pB37R, replacing the *gag* gene of HIV-1 (24). The resulting vector, pPABP1, contains the HIV-1 long terminal repeat (LTR) promoter. To generate a PABP1-GFP fusion protein, the coding sequence of PABP1 was inserted in the *Nhe*I site of plasmid pF25, which contains the mutant GFPsg25 gene linked to the immediate early cytomegalovirus (CMV) promoter (25). Deletion mutants of PABP1-GFP were generated by PCR. The corresponding primers also contained *Nhe*I restriction site, and resulting PCR fragments were inserted into pF25. Deletion mutants shown in Fig. 2 contained the following PABP1 coding sequences: M1, nt 1–255; M1', nt 1–450; M12, nt 1–555; M2, nt 270–555; M1234, nt 1–1125; M4c, nt 835–1499; and c, nt 1128–1499.

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¹ The abbreviations used are: PABP1, poly(A)-binding protein 1; GFP, green fluorescent protein; INS1, inhibitory/instability sequence 1; HIV-1, human immunodeficiency virus, type 1; LTR, long terminal repeat; nt, nucleotide(s); PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; RDR, rhodamine red; DRB, 5,6-dichlororibofuranosylbenzimidazole.

Cells and Transfections—HLtat is a HeLa-derived cell line that constitutively produces Tat protein (26). 293 is an adenovirus-transformed human embryonic kidney cell line (27). Cells were transfected by the calcium phosphate coprecipitation technique as described previously (28, 29). NIH 3T3 cells were transfected by LipofectAMINE (Life Technologies, Inc.).

A cell line constitutively expressing PABP1-GFP was generated by transfection of the PABP1-GFP-expressing plasmid into HeLa cells, followed by selection of G418-resistant clones. After a week of selection, cells expressing PABP1-GFP were sorted by FACS and maintained in G418-containing medium for 2 weeks, followed by a single-cell sorting procedure to generate clonal cell lines. Of three clones examined in detail, we report results obtained with clone F4.2. Analysis of protein expression in two clonal cell lines by Western blot using anti-PABP1 antiserum 39473 revealed that the level of expression of PABP1-GFP was less than 30% of the endogenous PABP1 in this clonal cell population. Therefore, the majority of the cells contain a small fraction of PABP1-GFP compared with the endogenous PABP1 protein.

Protein Analysis—Indirect immunofluorescence analysis was carried out as described previously (29). Briefly, cells were seeded into coated 50-mm glass bottom dishes (MatTek) and transfected 24 h later with the indicated plasmids. One day later, cells were fixed with 3.7% formaldehyde in PBS at room temperature for 15 min followed by incubation with 10% Nonidet P-40 in PBS for 15 min. Staining was performed by incubation with anti-PABP1 antiserum 39473 (1:50 dilution in PBS) for 40 min at 37 °C (20). The cells were then washed three times with PBS and incubated with FITC-conjugated anti-rabbit antibody (KRL, Rockville, MD) (1:20 dilution in PBS) for 40 min at 37 °C. For colocalization experiments, cells were incubated in addition to the anti-PABP1 antiserum with a mouse monoclonal anti-SC35 antibody (a gift from F. Maldarelli National Institutes of Health) followed by incubation with rhodamine red (RDR)-conjugated anti-mouse antibody (KRL). The cells were examined by an inverted fluorescence microscope (Zeiss Axiovert 135). GFP signals were obtained with an FITC-fluorescence filter set (Zeiss 09, excitation 450–490 nm, beam splitter 510 nm, emission filter >520 nm). 12-bit black and white images were captured using a digital CCD camera (Photometrics, AZ). Image analysis and presentation were performed using IPLab Spectrum software (Scanalytics, Vienna, VA). The total cellular GFP signal was measured by calculating the integrated pixel intensity in the imaged cell multiplied by the area of the cell. Nuclear signal was similarly obtained by measuring the pixel intensity in the nucleus. The cytoplasmic signal was calculated by subtracting the nuclear signal from the total cellular signal. All pixel values were measured below the saturation limits of the CCD camera. Analysis of PABP1 by immunoblotting was performed as described previously (20).

Cell Fusion and Heterokaryon Formation—HeLa cells transfected with pF25PABP1-GFP were seeded 24 h after transfection with a 10-fold excess of untransfected HeLa cells. After overnight incubation, the cells were treated with 100 μ g/ml of cycloheximide for 1 h, and then treated with pre-warmed 4% polyethylene glycol for 3 min (30). The cells were washed with medium containing 100 μ g/ml of cycloheximide and incubated for 1–5 h. Cells were observed under phase contrast and fluorescent illumination, and fusions involving one donor cell and surrounding acceptor cells were selected for analysis over a 5-h period. Serial CCD camera images were analyzed using IPLab Spectrum software.

RESULTS

PABP1 Is Found in the Nucleus After Transcription Inhibition—Although PABP1 is localized in the cytoplasm by immunofluorescence (Fig. 1A, panel 1) (23), several experiments have suggested a nuclear role for this protein (4, 9, 20). To examine a possible nuclear localization of PABP1, we tested whether localization of PABP1 depends on ongoing transcription since PABP1 is a mRNA-binding protein. For this, we treated cells with transcription inhibitor, 5,6-dichlororibofuranosylbenzimidazole (DRB) known to cause early termination of pre-mRNA transcription (Fig. 1A) (31). Treatment with DRB (25 μ g/ml) for 6 h, resulted in accumulation of PABP1 in the nucleus, mostly in speckles, as shown by immunofluorescence (Fig. 1A, panel 2). In addition, we constructed a HeLa-derived stable cell line expressing PABP1-GFP (Fig. 2) as described under “Materials and Methods.” In the great majority of these cells, PABP1-GFP was localized in the cytoplasm (Fig. 1B, panel 1), while in few cells we observed nuclear speckles containing PABP1-GFP.

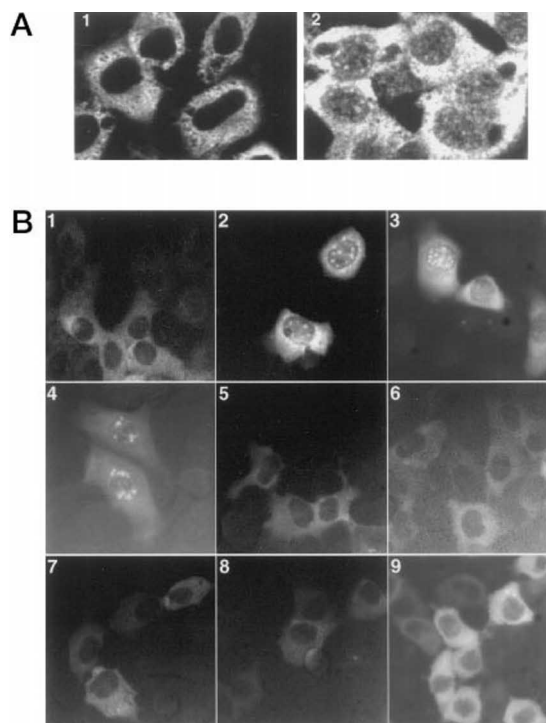


FIG. 1. Inhibition of transcription results in nuclear accumulation of PABP1. A, immunofluorescence analysis. HeLa cells were fixed and stained for indirect immunofluorescence using anti-PABP1 antiserum 39473 as described under “Materials and Methods.” 1, untreated cells; 2, cells treated with 25 μ g/ml of DRB for 6 h at 37 °C. B, CCD camera images of live HeLa cells (clone F4.2) stably expressing PABP1-GFP, treated for 4 h at 37 °C as follows: 1, no drug; 2, 0.5 μ g/ml of actinomycin D; 3, 5 μ g/ml of actinomycin D; 4, 25 μ g/ml of DRB; 5, cells treated with 25 μ g/ml of DRB for 4 h and then incubated in drug-free medium overnight; 6, cells treated with 100 μ g/ml of cycloheximide for 4 h; 7, cells treated with 25 μ g/ml of DRB, 4 h at 4 °C; 8, cells treated with 5 μ g/ml of actinomycin D, 4 h at 4 °C; 9, cells pretreated with glucose-free DMEM supplemented with 10 mM 2-deoxyglucose and 10 mM sodium azide for 1 h at 37 °C and then incubated with 25 μ g/ml of DRB, 4 h at 37 °C.

After 4 h of incubation of this clonal cell line with either DRB (25 μ g/ml) or with actinomycin D (0.5 μ g/ml or 5 μ g/ml), PABP1-GFP was observed in nuclear speckles (Fig. 1B, panels 2, 3, and 4). The DRB effect was reversible; after removing DRB and incubating the cells in fresh DRB-free medium, all PABP1-GFP was relocated to the cytoplasm (Fig. 1B, panel 5).

To test whether localization of PABP1 was dependent on ongoing translation, we examined the effect of the protein synthesis inhibitor cycloheximide on the subcellular distribution of PABP1 or PABP1-GFP in living cells. We did not observe any significant changes in the localization upon cycloheximide (100 μ g/ml) treatment alone (Fig. 1B, panel 6). To demonstrate complete inhibition of protein synthesis by cycloheximide under the conditions used in our experiments, we measured incorporation of radiolabeled amino acids into trichloroacetic acid (TCA)-insoluble protein fraction from untreated and cycloheximide-treated cells. The presence of cycloheximide inhibited protein synthesis by 97%.

These results suggested that PABP1 is able to enter the nucleus, and its exit to the cytoplasm is dependent on active RNA transcription. Given the size of the protein, we thought it is unlikely that PABP1 passively diffuses into the nucleus. To investigate whether the nuclear uptake of PABP1 is an energy-dependent process, we treated cells with actinomycin D or with DRB at 4 °C. PABP1-GFP failed to accumulate in the nucleus under these conditions (Fig. 1B, panels 7 and 8). Import of PABP1 was also abolished in the cells upon inhibition of tran-

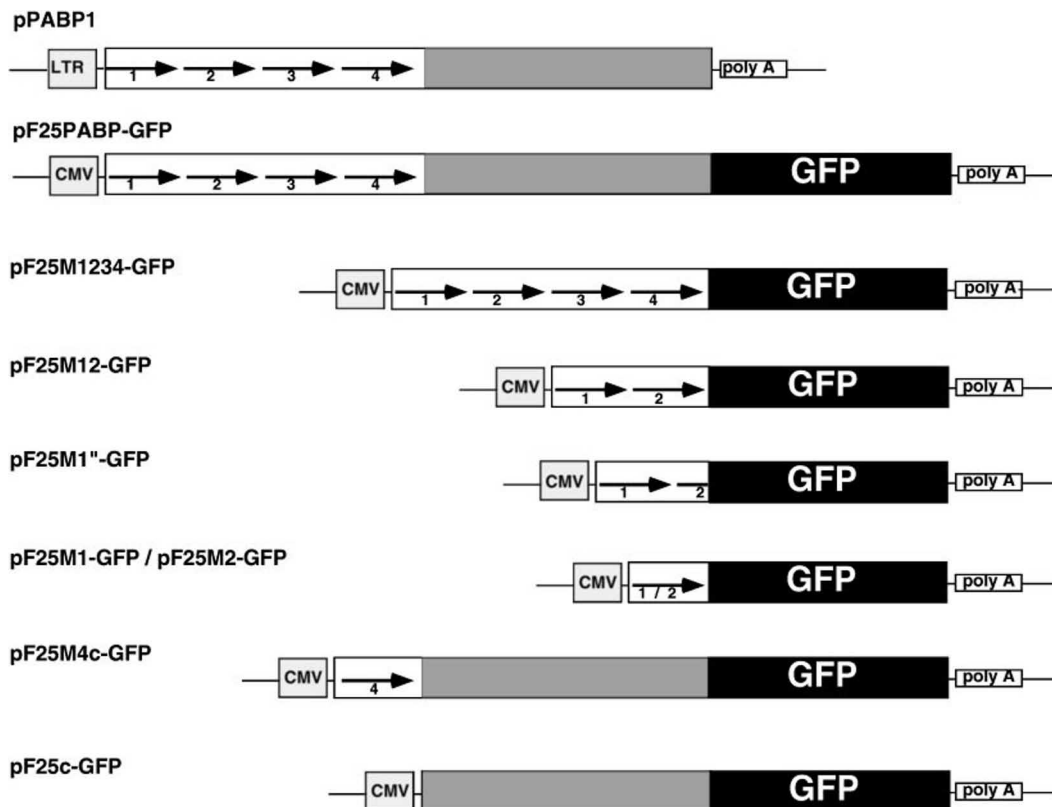


FIG. 2. **Expression vectors for PABP1 and mutants.** Plasmids expressing PABP1, PABP1-GFP, and mutants of PABP1 fused to GFP coding sequence are shown, with arrows corresponding to the PABP1 RNA-binding domains (RBDs) and gray areas to the C-terminal part of the protein. LTR, HIV-1 LTR promoter; CMV, cytomegalovirus early promoter; poly(A), polyadenylation signal.

scription in the presence of 2-deoxyglucose (10 mM) and sodium azide (10 mM) that deplete ATP pools (32–34) (Fig. 1B, panel 9). Administration of sodium azide alone did not completely prevent the accumulation of the PABP1 in the nucleus (data not shown). We interpret the results of these experiments to suggest that the import of PABP1 to the nucleus is an energy-requiring process.

PABP1 Localizes in Both the Cytoplasm and Nucleus upon Transfection—We studied the localization of PABP1 in HeLa cells upon transient transfection of plasmids expressing a full-length PABP1 cDNA (pPABP1, Fig. 2). Indirect immunofluorescence showed that PABP1 accumulated in the nucleus of cells producing high levels of the protein, whereas PABP1 showed cytoplasmic localization in cells expressing low levels of protein or in non-transfected cells (Fig. 3, A and B). We also introduced GFP-tagged PABP1 protein into the cells by transient transfections of pF25PABP1-GFP, a plasmid producing a PABP1-GFP fusion protein (Fig. 2). Similarly to transfected PABP1, PABP1-GFP was found to have a variable distribution in transfected cells. In low expressing cells, PABP1-GFP was found in the cytoplasm (Fig. 3D), whereas high expressing cells displayed nuclear accumulation of the fusion protein (Fig. 3C). Similar results were obtained upon transfection of human kidney 293 cells and mouse NIH-3T3 cells (data not shown). Comparison of the levels of expression of PABP1-GFP in cells containing nuclear/cytoplasmic *versus* cytoplasmic only PABP1-GFP was performed by quantitation of GFP fluorescence. Series of images of transfected cells were taken at identical settings and total GFP signal was measured as described under “Materials and Methods,” below the saturation limits of the CCD camera. The fluorescence levels of cells containing PABP1-GFP only in the cytoplasm was on average 3–5-fold lower than that of cells containing PABP1-GFP both in the cytoplasm and nucleus.

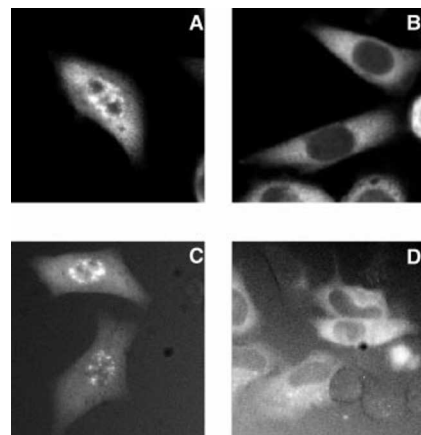


FIG. 3. **Nuclear accumulation of PABP1 or PABP1-GFP upon transient transfection.** HLtat cells were transiently transfected with 3 μ g of the PABP1-expressing plasmid pPABP1 (A and B) or with the PABP1-GFP-expressing plasmid pF25PABP1-GFP (C and D). Detection of PABP1 in panels A and B was by immunofluorescence as in Fig. 1A above, whereas detection of PABP1-GFP in live cells was by excitation using blue light. PABP1 (A) or PABP1-GFP (C) accumulated in the nucleus of high-expressing cells. Cells expressing low levels of PABP1 (B) or PABP1-GFP (D) showed cytoplasmic localization of the protein. Representative fields are shown. Exposure times for images (B) and (D) were longer compared with (A) and (C).

In most cells containing nuclear PABP1, the protein accumulated in nuclear speckles. This was observed both in live cells expressing PABP1-GFP and in fixed cells expressing untagged PABP1 (Fig. 4, A and D). Because this localization was reminiscent of the nuclear speckles containing splicing factors, the cells were immunostained with antiserum against the splicing factor SC35 to further characterize the intranuclear location of PABP1 (Fig. 4, C and F). Combining the signal from

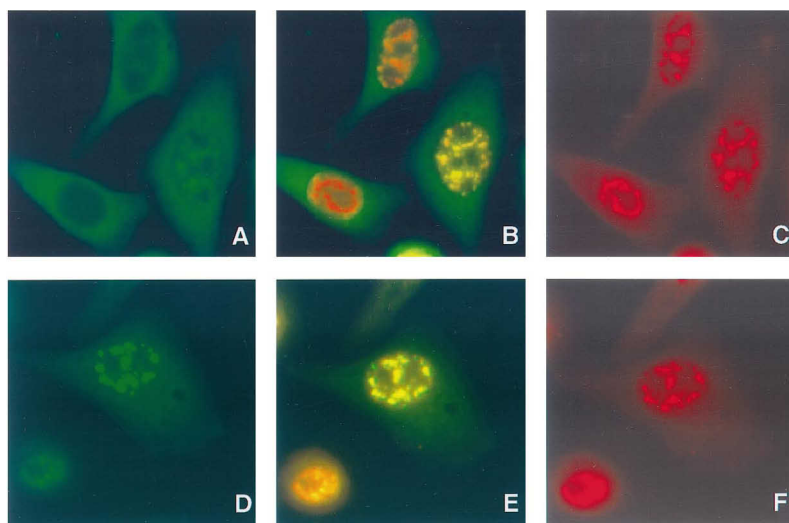


FIG. 4. Colocalization of PABP1 or PABP1-GFP and SC35 in nuclear speckles. HLtat cells were transiently transfected with 1 μ g of a PABP1-expressing plasmid (*top panels*) or PABP1-GFP-expressing plasmid (*bottom panels*). At 24-h post-transfection, cells were fixed, immunostained, and examined by fluorescent microscopy as described under "Materials and Methods." Cells at the *top panel* were subjected to double immunostaining with rabbit antiserum against PABP1 and FITC-conjugated anti-rabbit antibody together with mouse anti-SC35 monoclonal antibody and RDR-conjugated anti-mouse antibody. Cells at the *bottom panel* were stained with mouse anti-SC35 monoclonal antibody and RDR-conjugated anti-mouse antibody. PABP1-GFP was detected directly using the FITC filter set. *A*, PABP1 localization; *C*, SC35 localization; *D*, PABP1-GFP detection; *F*, SC35 localization; *B* and *E*, combined images for FITC and RDR fluorescence show colocalization of PABP1 and SC35.

PABP1-GFP with the corresponding immunostaining image of SC35 or double immunostaining of cells transfected with PABP1 revealed significant colocalization of PABP1 and SC35 nuclear speckles (Fig. 4, *B* and *E*).

These results suggested that PABP1 may traffic between the nucleus and the cytoplasm. Another way to study nuclear-cytoplasmic transport is to utilize heterokaryon assay upon cell fusion similar to previously published procedures (35, 36). Heterokaryon formation temporarily creates a nonequilibrium situation, allowing real-time study of protein redistribution among different intracellular compartments. This assay allows simultaneous monitoring of the exit of the protein of interest from the donor nucleus to the cytoplasm and the import into the surrounding acceptor nuclei. HeLa cells containing PABP1-GFP in the nucleus were fused to excess of untransfected cells. The localization of PABP1-GFP was followed in living cells after cell fusion. Cycloheximide (100 μ g/ml) was added to prevent *de novo* protein synthesis. Fig. 5*A* shows the movement of PABP1-GFP over a period of 4.5 h in two independent heterokaryons initially containing one bright nucleus and either one or two empty nuclei. We were able to detect PABP1-GFP in the acceptor (untransfected) nuclei within 1–2 h after fusion. We also observed a decrease in the intensity of fluorescence in the donor nucleus in parallel to the increase in the acceptor nuclei (Fig. 5*A*, compare donor nuclei of both fusions at 1- and 4.5-h time points). Quantitation of the fluorescence signal revealed that the ratio of the total nuclear signal to the cytoplasmic signal for the whole heterokaryon was not changed over this period, whereas the signal within the donor nucleus decreased and that of the acceptor nuclei increased (Fig. 5*B*). Therefore, PABP1-GFP migrated out of the donor nucleus and was imported into the acceptor nuclei.

The Two N-terminal RNA-binding Domains of PABP1 Are Sufficient for Nuclear Localization—PABP1 contains four RNA-binding domains (RBDs) and a proline-rich C-terminal domain (Fig. 2). To investigate the part of PABP1 responsible for nuclear accumulation, we generated deletion mutants coupled to GFP and introduced them into HeLa cells by transient transfection (Fig. 2). Interestingly, deletion mutants containing the two N-terminal RBDs (M12) or all four RBDs (M1234) of PABP1 showed predominantly nuclear localization (Fig. 6, *A*

and *B*). In contrast to PABP1-GFP, we did not observe any significant differences in the intracellular distribution of these mutant proteins associated with different levels of expression in individual transfected cells.

Mutants containing single RBDs (M1 or M2; Fig. 6, *C* and *E*, respectively) or only the C-terminal part of PABP1 (Fig. 6*G*) were distributed throughout the entire cell, and their localization was indistinguishable from that of GFP alone (Fig. 6*H*). Mutants containing the first RBD and part of the second domain (M1', Fig. 6*D*) or the fourth RBD and the C-terminal part of PABP1 (M4c, Fig. 6*F*) were also distributed throughout the entire cell. This distribution of GFP hybrids containing small fragments of PABP1 may reflect passive diffusion among cellular compartments. We conclude that mutant M12 contains the minimal part of PABP1 necessary and sufficient for nuclear localization.

We next investigated the ability of the mutant polypeptides to bind poly(A) *in vitro*. Cell lysates from cells transfected with DNA expressing the different mutants were incubated with poly(A)-Sepharose, and the presence of bound PABP1 or mutant polypeptides was examined by immunoblotting after extensive washing with 1 M KCl (Fig. 7). Both PABP1 and PABP1-GFP bound tightly to poly(A), and were retained on the poly(A)-Sepharose matrix after this wash step. The mutants M12 and M1234 also bound efficiently to poly(A). A significant amount of each polypeptide was retained on the poly(A)-Sepharose beads after the wash step with 1 M KCl (Fig. 7, *lanes* 2, 6, 8, and 14). We did not observe any significant poly(A) binding activity with either M1, c (the C-terminal fragment of PABP1), or M4c (Fig. 7, *lanes* 4, 10, and 12). The decrease of the intensity of the retained band corresponding to these mutant polypeptides could not be attributed to the degradation of the protein during the experiment since we were able to detect the unbound mutant polypeptides in the flow-through fractions (data not shown). These results are in agreement with the results of Burd *et al.* (37) showing that a contiguous two RBD combination is required for efficient RNA binding. As shown above, none of the single-domain polypeptides were able to be retained in the nucleus, suggesting a correlation between nuclear accumulation and the ability of the polypeptides to bind tightly to poly(A) RNA.

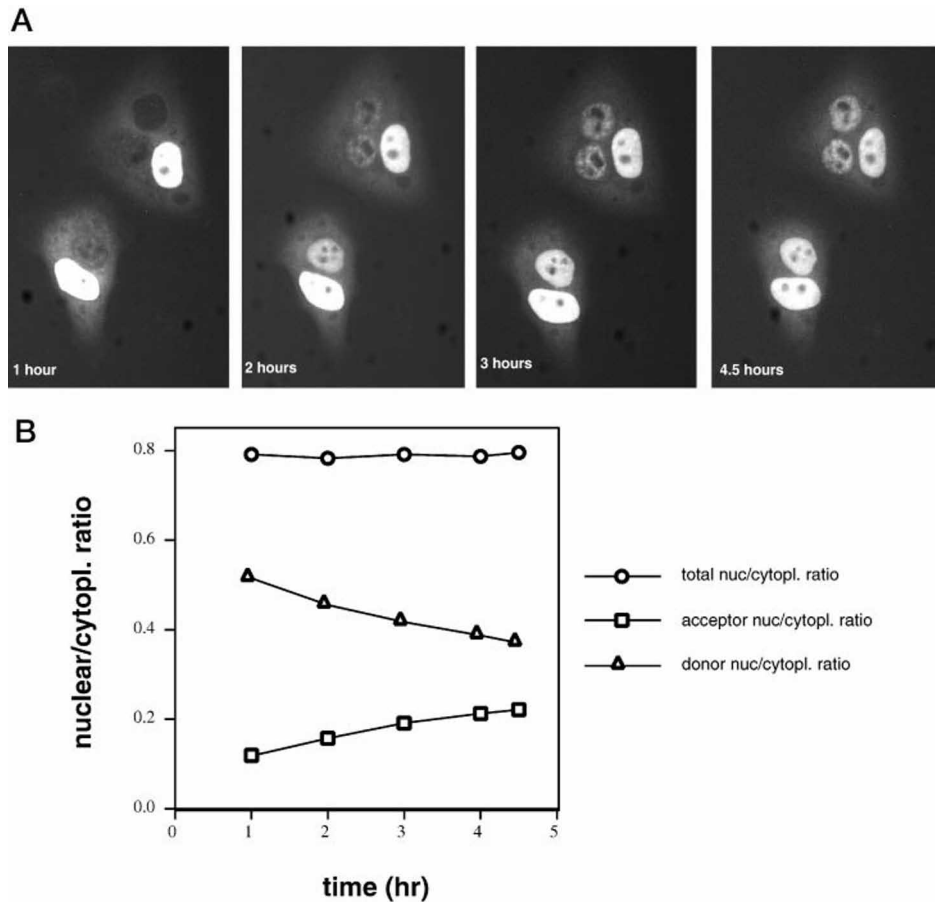


FIG. 5. Shuttling of PABP1 after cell fusion. **A**, redistribution of PABP1-GFP from donor nuclei into acceptor nuclei 1–4.5 h after PEG-mediated fusion. HLtat cells were transfected with 3 μ g of PABP1-GFP-expressing plasmid. PABP1-GFP-expressing cells were cocultivated with excess of untransfected HLtat cells, and cell fusion was performed the next day as described under “Materials and Methods.” Two independent heterokaryons in the same field are shown. **B**, quantitation of nuclear/cytoplasmic signal ratios for PABP1-GFP within the heterokaryon in panel **A**, over time. *Circles*, total nuclear/cytoplasmic signal; *triangles*, donor nucleus/cytoplasmic signal; *squares*, acceptor nucleus/cytoplasmic signal ratios. GFP fluorescence was quantified from the serial CCD camera images as described under “Materials and Methods.”

DISCUSSION

In this study, we present evidence that the cytoplasmic localization of PABP1 represents a dynamic equilibrium of the protein, which has the ability to shuttle between the nucleus and cytoplasm. Different types of evidence support this conclusion. First, inhibition of transcription results in PABP1 accumulation in nuclear speckles. Removal of the inhibitor (DRB) restores the cytoplasmic localization of PABP1. Second, overexpression of the protein results also in nuclear speckle accumulation. We have performed analysis of either PABP1 or PABP1-GFP localization and trafficking. In all cases, the results were in agreement, indicating that GFP tagging did not affect PABP1 function. We show that the import of PABP1 is an active process, since PABP1 fails to enter the nuclei upon ATP depletion or at low temperature. We observed that two deletion mutants of PABP1 containing two or four RBDs have retained the ability to enter the nucleus. In agreement with our data, a truncated form of PABP1 encompassing only four RBDs, which is very similar to our M1234 mutant, was previously found in the nucleus (4). These mutants, M12 and M1234, readily interacted with poly(A) RNA with an affinity comparable with that of the full-length protein. Deletion mutants exhibiting reduced RNA binding activity did not show any preference in localization. Therefore, the minimal putative nuclear localization signal of PABP1 is composed of two RBDs and was not associated with any short peptide sequence. RNA binding and nuclear import may be distinct, and RNA binding may be the cause for nuclear retention. Such mechanism has been proposed for the Rev protein of HIV-1, based on the demonstration of nuclear import but not nuclear retention of Rev mutants that lack RNA

binding.² Previously, it was reported that PABP1 is localized exclusively to the cytoplasm, even upon overexpression of hemagglutinin-tagged PABP1 (17, 23). We do not know the reason for this discrepancy although it might reflect differences in the levels of expression, the type of cells used for transfection, or accessibility of the hemagglutinin tag to the antibodies. Similar to our results, Sachs (4) *et al.* have suggested the presence of nuclear PABP1 in yeast.

Nuclear PABP1 was concentrated in speckles that also contained splicing factor SC35. Speckles containing the splicing factors SC35, Sm, and U2AF were initially thought to be a factor storage compartment (38–42). Others have suggested a more dynamic functional role for nuclear speckles, due to their marked reorganization upon cell differentiation or inhibition of transcription (43–48). Huang and Spector (43) reported colocalization of poly(A)-containing RNA and SC35 speckles and reorganization of such structures upon inhibition of transcription, suggesting an active role in the RNA processing itself. Therefore, we suggest that the binding of PABP1 to poly(A)-containing RNA occurs in the nuclear speckles during or shortly after transcription.

Since PABP1 accumulated in the nucleus upon overexpression, we hypothesize that the export pathway of the protein is saturable. To explore the possible mechanism of PABP1 export, we investigated its dependence on the inhibition of transcription and translation. Transcription inhibition resulted in accumulation of PABP1 in the nuclei of the cells expressing low levels of PABP1. Inhibition of translation by cycloheximide did

² R. Stauber, E. Afonina, and G. N. Pavlakis, unpublished data.

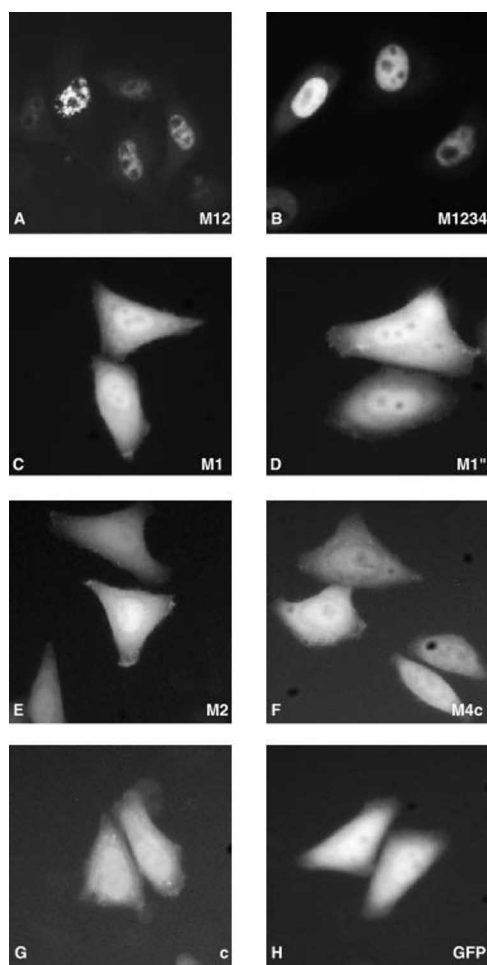


FIG. 6. Cellular localization of PABP1-GFP mutants. HLtat cells were transfected with 1–3 μ g of indicated plasmids. At 24 h post-transfection, fluorescent microscopy was performed. Representative fields of live cells captured by CCD camera are shown.

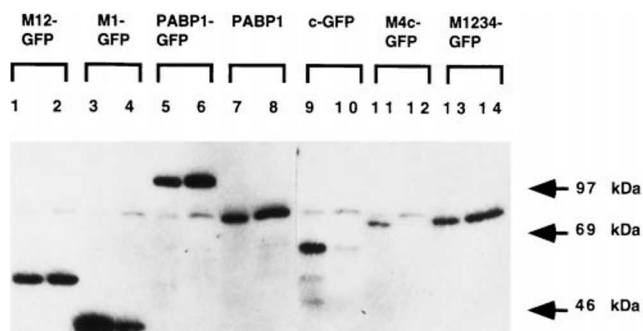


FIG. 7. Comparison of poly(A) binding activity of the PABP1, PABP1-GFP, and mutants. Binding of PABP1 and mutants to poly(A) *in vitro* measured after immunoblotting of the proteins captured on poly(A) beads. HLtat cells were transfected with the indicated plasmids expressing PABP1 and mutants. A 50- μ l aliquot of cell extract from each transfection was loaded in lanes 1, 3, 5, 7, 9, 11, and 13. A 200- μ l aliquot of cell extract was bound to poly(A)-Sepharose beads, washed with 1 M KCl, and the bound proteins were boiled in loading buffer containing SDS and loaded on lanes 2, 4, 6, 8, 10, 12, and 14, respectively.

not affect the intracellular distribution of PABP1. The requirement for ongoing transcription for PABP1 export suggests that PABP1 utilizes its RNA binding activity to exit the nucleus as RNP particles. mRNA export has been suggested to be mediated by hnRNP proteins. Of these, the best characterized candidate is hnRNP A1, which is constantly shuttling between

nucleus and cytoplasm (17–19). Future experiments are required to investigate whether PABP1 and hnRNP proteins utilize the same export pathway.

The trafficking pattern identified for PABP1 may be shared with other proteins. We have recently shown that the product of the von Hippel-Lindau tumor suppressor gene (*pVHL*) (49–52) has trafficking properties similar to PABP1, *i.e.* *pVHL* shuttles between the nucleus and cytoplasm and, similar to PABP1, inhibition of transcription resulted in nuclear accumulation.³ The similarity between the intracellular localization changes and trafficking pattern for PABP1 and *pVHL* suggests similar trafficking mechanisms for the two proteins.

The suggestion that PABP1 has the ability to interact with poly(A)-containing RNA in the nucleus and that it exits the nucleus as a complex with newly synthesized poly(A)-containing RNA raises the possibility that PABP1 binding to the poly(A) tail in the nucleus is important for mRNA processing, transport, and utilization. Recently, PABP1 was identified as a component of CF I complex responsible for pre-messenger mRNA 3'-end formation (9). Thus, PABP1 is involved in early steps of regulation of mRNA expression that occur in the nucleus. It was hypothesized that PABP1 binds to progressively growing poly(A) tail and that the amount of bound PABP1 regulates poly(A)-polymerase activity. The yeast PABP1 homologue Pab1p was also reported to interact with the cap-binding complex via the eIF-4G subunit (16). It is tempting to speculate that mRNA is transported out of the nucleus as an RNP complex competent for translation initiation.

We have previously shown (20) that PABP1 binds the INS1 sequences within the HIV-1 mRNA and may participate in down-regulation of this mRNA. The proposed mechanism of this inhibition included the formation of aberrant complexes of PABP1 and the adenosine-rich regions of INS1. Our present finding that PABP1 is capable of trafficking between cellular compartments suggests that the complexes of PABP1 and INS1-containing RNA may form in the nucleus, which is consistent with the conclusion that INS acts in the nucleus. Incorrect binding of PABP1 within the mRNA may affect efficient RNP formation and export of this mRNA.

PABP1 is clearly a multifunctional protein involved in several steps of mRNA metabolism. Our results suggest that, by binding to the poly(A)-containing mRNA in the nucleus, PABP1 may participate in mRNP formation, transport, and utilization. Association of PABP1 with poly(A) RNA in the nucleus and transport of the protein as part of an RNP particle is an attractive hypothesis explaining different aspects of PABP1 function, and especially participation in mRNP maturation.

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