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Nuclear localization signals also mediate the outward movement of proteins from the nucleus

(shuttle/steroid hormone receptors/simian virus 40 large tumor antigen/nuclear export)

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ABSTRACT **Several nuclear proteins, including steroid hormone receptors, have been shown to shuttle continuously between the nucleus and the cytoplasm. The mechanism of entry of proteins into the nucleus is well documented, whereas the mechanism of their outward movement into the cytoplasm is not understood. We have grafted the nuclear localization signals of the progesterone receptor or the simian virus 40 large tumor antigen onto β-galactosidase. These additions were shown to impart to the protein the ability to shuttle between the nucleus and the cytoplasm. Microinjected proteins devoid of a nuclear localization signal were unable to exit from the nucleus. The same nuclear localization signals are thus involved in both the inward and the outward movement of proteins through the nuclear membrane. We also show that although the nuclear import requires energy, the nuclear export does not. These results suggest that the nucleocytoplasmic shuttling may be a general phenomenon for nuclear proteins that could possibly undergo modifications in the cytoplasm and exert some biological activities there. These conclusions also imply that at least part of the cellular machinery involved in the nuclear import of proteins may function bidirectionally.**

The mechanism by which nuclear proteins, synthesized in the cytoplasm, enter the nucleus has recently been extensively studied (for review, see refs. 1 and 2). Nuclear localization signals (NLSs) have been described that direct the proteins to the cytoplasmic side of the nuclear pore and are, thereafter, involved in their energy-dependent transfer inside the nucleus. These karyophilic signals are composed of a single stretch of basic amino acids [for instance, in the simian virus 40 (SV40) large tumor (T) antigen] (3, 4), are bipartite (as in the case of nucleoplasmin) (5), or are composed of four successive clusters of basic amino acids [as observed in the progesterone receptor (PR)] (ref. 6; A.G.-M. and E.M., unpublished data). These signals when conjugated to a cytoplasmic protein are sufficient to localize the latter in the nucleus. For some nucleolar proteins (7, 8) and steroid hormone receptors (9–12), it has been established that their nuclear localization actually reflects a dynamic situation, the protein continuously shuttling between the nucleus and the cytoplasm. We (9) and others (4, 13, 14) have shown that nonnuclear proteins when microinjected into the nucleus are unable to gain access to the cytoplasm. These observations, which, however, have been the subject of a recent controversy (15, 16), suggested that a protein needs a specific signal to be able to cross the nuclear membrane in the outward direction. The nature of this nuclear export signal, operating during protein nucleocytoplasmic shuttling, is unknown. By using deletion mutants of the PR, we have observed (refs. 9 and 17; A.G.-M. and E.M., unpublished data) a close correlation between the nuclear localization of the various mutants and their ability to shuttle. We thus wondered whether the NLS of the PR could be involved in both the inward and the outward transport of the protein.

MATERIALS AND METHODS

Plasmids. The expression vector pKSVβ-gal was obtained by ligating the *Sma* I–*Pst* I fragment encoding all but the first 7 amino acid residues of β-galactosidase (pMC1871, Pharmacia), with the *Xho* I–*Xho* I fragment of pKSV-rPR (18). This vector encodes a fusion protein containing the first 102 amino acids of the rabbit PR (rPR) [including the epitope recognized by monoclonal antibody (mAb) Let 126 (19)] fused with *Escherichia coli* β-galactosidase. The 102 N-terminal amino acids of the rPR have no effect on the compartmentalization of proteins (ref. 9; A.G.-M. and E.M., unpublished data). This construct is placed under the control of the SV40 early promoter. The fusion protein bearing the entire C-terminal region of β-galactosidase is expressed as a tetramer (20) and displays enzymatic activities. A unique *Bgl* II restriction site was obtained by ligating a *Bgl* II linker between the rPR fragment and the β-galactosidase.

The expression vector nlsPR-β-galactosidase was obtained by inserting a synthetic nlsPR corresponding to amino acids 593–653 of the rPR at this unique *Bgl* II site. β-Galactosidase produced with this construct was detected in the nucleus by immunocytochemistry and through its enzymatic activity.

The expression vector nlsSV40-β-galactosidase was obtained by inserting a synthetic sequence encoding amino acids PKKKRKV responsible for the nuclear localization of SV40 large T antigen (3) into the same *Bgl* II site.

Cell Cultures, Permanent Cell Lines, and Inhibitors of ATP Synthesis. Mouse L cells were cotransfected with the plasmid encoding nlsPR-β-galactosidase or nlsSV40-β-galactosidase and with the pSV-neo plasmid conferring resistance to Geneticin. Clones resistant to Geneticin (Sigma) were selected and screened for expression of β-galactosidase by immunocytochemistry (see below). The generation of mouse L cells permanently expressing the rPR has been described (9). Simian COS-7 cells, human 293 cells, and mouse L cells were grown as described (9). Sodium azide (10 mM), oligomycin (50 μM), or antimycin A (25 μM) was added to glucose-minus Dulbecco's modified Eagle's medium supplemented with 6 mM 2-deoxyglucose as described (9).

Immunofluorescence Studies and *in Situ* Detection of β-Galactosidase Activity. Immunofluorescence studies were performed essentially as described (17). For the detection of β-galactosidase antigenicity, the cells were fixed with paraformaldehyde [4% (wt/vol) in phosphate-buffered saline (PBS)] and permeabilized with Triton X-100 (0.5% in PBS). An anti-β-galactosidase mAb (Boehringer Mannheim) was

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Abbreviations: NLS, nuclear localization signal; PR, progesterone receptor; rPR, rabbit PR; SV40, simian virus 40; T, tumor; mAb, monoclonal antibody.

used at 10 μg/ml. For the detection of the first 102 amino acids of the fusion protein, mAb Let 126 (19) was used at 15 μg/ml. A fluorescein- or rhodamin-conjugated rabbit anti-mouse antibody (Dakopatts, Glostrup, Denmark) was added at a dilution of 1:40. *In situ* β-galactosidase activity was detected as described (21) after fixation in 0.5% glutaraldehyde.

Heterokaryon Formation. Fusions were carried out as described (8, 10). Human 293 cells or mouse L cells were plated on chamber slides (Nunc). Twenty-four hours later, L cells expressing the rPR or COS-7 cells, respectively, were plated onto the same chamber slides. The cells were fused 6 h later. Cycloheximide (10 μg/ml) was added immediately after the fusion as described (9, 22). This concentration suppressed 95% of protein synthesis (refs. 23 and 24; K.D. and A.G.-M., unpublished data). The cells were fixed at various times after the fusion (4 h, 6 h, 16 h, or 24 h). In some cases, higher concentrations (100 μg/ml) of cycloheximide were used during 4-h incubations. Immunofluorescence studies of β-galactosidase distribution were performed as described above. The same result was obtained with anti-β-galactosidase or Let 126 mAb. For the detection of the large T antigen, an anti-large T antigen mAb and a fluorescein-conjugated rabbit anti-mouse secondary antibody were used. For staining of DNA, Hoechst 33258 (Sigma) was added at 1 μg/ml at the time of incubation with the secondary antibody. Mouse L-cell nuclei were characterized by the fluorescence of their satellite DNA (7, 9).

Microinjection Experiments. L cells were plated onto 35-mm dishes (Nunc). Twenty-four hours later, cell nuclei were microinjected with a solution of β-galactosidase (Sigma) (10 mg/ml in 2 mM Pipes/140 mM KCl, pH 7.4). Fluorescein-conjugated dextran (Sigma) was also added to monitor the microinjection of nuclei. The microinjection procedure was as described (9). The cells were then incubated at 37°C in sodium azide or in standard medium 4 h prior to fixation. The immunocytochemical detection of β-galactosidase was performed as described above.

Western Blot Studies. Transfections were performed by calcium phosphate coprecipitation (18) with plasmid DNA encoding the rPR (pKSV-rPR) or nlsSV40-β-galactosidase. Cells were collected in ice-cold PBS 40 h after transfection and centrifuged at 4°C at 120 × *g*. After one wash in ice-cold PBS, the cell pellets were homogenized in a Teflon–glass Potter–Elvehjem apparatus at a cell/buffer ratio of 1:3 (wt/vol) in TES (1.5 mM EDTA/0.25 M sucrose/10 mM Tris·HCl, pH 7.4) containing a mixture of proteolysis inhibitors as described (25). Cytosols were obtained by a 100,000 × *g* centrifugation of the homogenates for 65 min at 4°C (25). The pellets containing the nuclei were washed twice in TES by resuspension and centrifugation at 1500 × *g*. The two supernatants were pooled, ultracentrifuged at 100,000 × *g* for 65 min, and stored ("wash"). The nuclei were extracted for 30 min at the same cell/buffer ratio with TES supplemented with 0.4 M KCl and then frozen. After thawing, the nuclear extracts were isolated by ultracentrifugation at 100,000 × *g* for 30 min. Equivalent aliquots of all the fractions were electrophoresed in denaturing conditions in a 7% polyacrylamide gel (26). Immunoblot analysis was performed as described (25) with the Let 126 anti-receptor mAb (3 μg/ml). A sheep anti-mouse horseradish peroxidase-linked antibody (Amersham) was used at a 1:4000 dilution. The immunoglobulins were revealed with the ECL procedure (Amersham).

RESULTS AND DISCUSSION

Nucleocytoplasmic Shuttling of nlsPR-β-Galactosidase. To study the role of the NLS of the PR on the export of the protein, we constructed an expression vector encoding the bacterial β-galactosidase to which was added the NLS of the PR (nlsPR-β-galactosidase). We have shown (9) that, in the shuttling process of the PR, the inward movement needs energy, whereas the outward movement does not. Thus administration of ATP synthesis inhibitors leads to an efflux of the receptor from the nucleus into the cytoplasm. We thus prepared an L-cell line that permanently expresses the nlsPR-β-galactosidase construct and observed, as expected, a nuclear localization of the β-galactosidase antigen (Fig. 1*A*). Administration of sodium azide provoked an efflux from the nucleus of the β-galactosidase, which then accumulated in the cytoplasm (Fig. 1*B*). As described (9), this effect was not due to a nonspecific action of sodium azide since it was observed with a variety of inhibitors of energy synthesis (oligomycin, antimycin A, etc.) having different mechanisms of action (data not shown). The effect of sodium azide was not due to irreversible cell damage: in cells transferred back into medium devoid of the inhibitor, the receptor regained its initial localization in the nucleus (Fig. 1*C*). Such a reversibility of action seen with inhibitors of energy synthesis has previously been shown for the intracellular localization of the full-length PR (9). These observations were not cell-dependent since an identical effect of sodium azide was observed in COS-7 cells transfected with the nlsPR-β-galactosidase construct (data not shown). If β-galactosidase itself (devoid of any NLS) was microinjected into L-cell nuclei, the administration of sodium azide was without effect, in this case the protein remained inside the nucleus (Fig. 1*E*). This experiment confirmed previous results involving microinjection of immunoglobulins (9) and more recent data obtained by microinjection of albumin (data not shown).

One of the most illustrative ways previously used to show the existence of a nucleocytoplasmic shuttling process has been the production of heterokaryons by fusing cells expressing a certain nuclear protein with other cells devoid of thi-

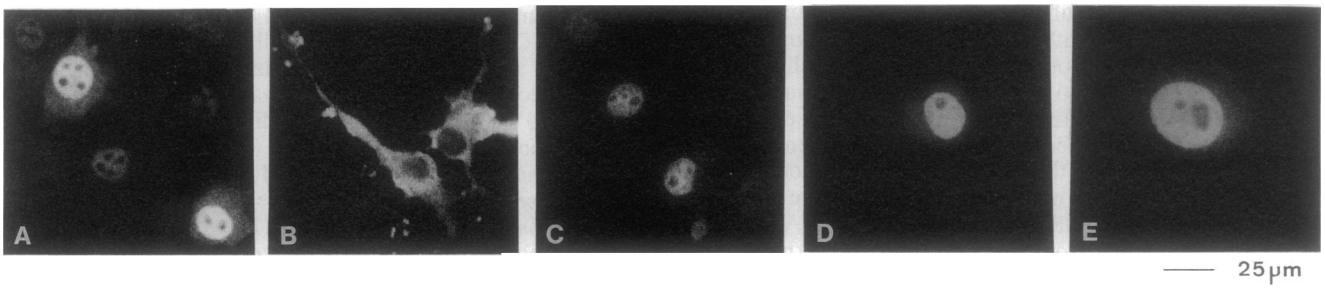

FIG. 1. Energy depletion provokes the efflux of nuclear nlsPR-β-galactosidase into the cytoplasm. In an L-cell line permanently expressing nlsPR-β-galactosidase construct, β-galactosidase was localized in the nucleus (*A*). The cells were then cultured in the presence of 2-deoxyglucose and sodium azide (10 mM) for 4 h. In these conditions, β-galactosidase was shifted into the cytoplasm (*B*). (The same result was observed whether cycloheximide at 10 μg/ml was present or not.) The cells were finally transferred into a medium containing glucose and devoid of sodium azide. After 4 h of incubation, β-galactosidase was again detected in the nucleus (*C*). β-Galactosidase (without added NLS) microinjected into mouse L-cell nuclei was located in the nucleus (*D*). This compartmentalization was not affected by sodium azide (*E*).

protein. The transfer of the protein from one nucleus to another, in conditions where the neosynthesis of the protein is blocked, demonstrates the transit of the protein through the cytoplasm (7, 9, 22). We thus fused the L cells that permanently express nlsPR-β-galactosidase with human kidney 293 cells. The mouse nuclei were easily identified in heterokaryons by the presence of a characteristic satellite DNA detected by Hoechst 33258 (7). In conditions where protein synthesis was suppressed by cycloheximide, the β-galactosidase antigen was transferred from L-cell nuclei into human-cell nuclei, thus, showing the existence of a shuttling mechanism (Fig. 2).

Nucleocytoplasmic Shuttling of nlsSV40-β-Galactosidase. We then wondered whether the NLS of the PR had any distinct property or whether other karyophilic signals could behave similarly. We thus used another construct in which the NLS of SV40 large T antigen was linked to β-galactosidase (nlsSV40-β-galactosidase). We next prepared an L-cell line that permanently expressed the nlsSV40-β-galactosidase construct. The β-galactosidase antigen was observed in the nucleus (Fig. 3*A*). When sodium azide was added to the cells, the β-galactosidase was transferred into the cytoplasm (Fig. 3*B*). It was again verified that the effect of sodium azide was reversible (Fig. 3*C*). A fusion experiment was then performed between the L-cell line expressing nlsSV40-β-galactosidase and the human 293 cells. Again transfer of β-galactosidase was observed from L-cell nuclei toward the nuclei originating from the 293 cells (Fig. 3*D*).

Nuclear Proteins Carrying Localization Signals and Not Bound to Nuclear Constituents Are Recovered in the Cytosol of Cell Homogenates. In the study of the cellular localization of steroid hormone receptors, one of the most striking features, which led to many controversies, was the apparent contradiction between results of cell fractionation studies (27, 28) and *in situ* localization of proteins (29–32). In the absence of hormone, after tissue homogenization at 0–4°C, in conditions where the nuclear membrane is conserved, the receptor is recovered in the cytosol (27, 28). For most of these receptors and especially for sex steroid receptors, immunocytochemistry and cell enucleation show the protein to be intranuclear

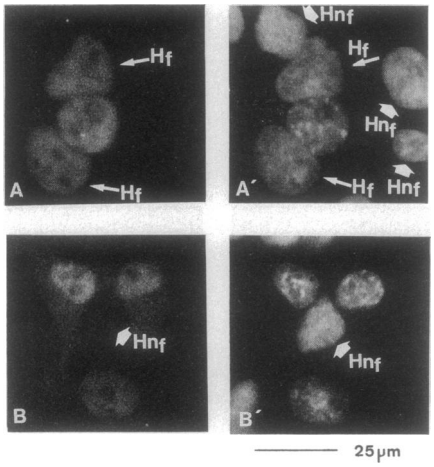

FIG. 2. Transfer of nlsPR-β-galactosidase from mouse to human nuclei in heterokaryons. (*A* and *A'*) Mouse L cells expressing nlsPR-β-galactosidase were fused with 293 human cells and the heterokaryons were observed after 16 h. Cycloheximide was added immediately after the fusion as described (9, 22). β-Galactosidase appears in the two human nuclei included in the heterokaryon (Hf = human, fused). Note the absence of β-galactosidase in human nuclei not included in the heterokaryons (Hnf = human, nonfused). Unmarked nuclei belong to mouse cells. (*B* and *B'*) Control experiment in which adjacent cells have not been fused. The thick arrow indicates a human nucleus (Hnf). (*A* and *B*) Immunolocalization of β-galactosidase. (*A'* and *B'*) Hoechst 33258 fluorescence was used to visualize DNA.

(29–32). We have recently proposed that a likely explanation for this discrepancy that after homogenization, at low temperature and in diluting conditions, the energy-dependent nuclear import is prevented whereas the outward diffusion is still functional (9). If the latter is mediated by NLSs, this phenomenon should also occur for nonnuclear proteins to which such a signal has been experimentally attached. We thus homogenized COS-7 cells transfected with an expression vector encoding the PR [pKSV-rPR (18)] or with nlsSV40-β-galactosidase and prepared cytosols and nuclei. The latter were extracted with a high ionic strength buffer. Western blots (Fig. 4) showed that most of nlsSV40-β-galactosidase was recovered in the cytosol, as was most of the PR. The same redistribution after cell homogenization was observed for nlsPR-β-galactosidase (data not shown). If "leakage" of nuclear proteins is due to a NLS-mediated outward diffusion, it should be a general phenomenon not restricted to steroid hormone receptors. Indeed, it has been observed that >50% of nuclear proteins are lost from the nuclei in 10 min (33). More specifically, myc (34), myb (34), DNA ligase I (35), and nucleolar protein Nopp 140 (36) were recovered in the cytosol after cell homogenization.

All these experiments thus showed that a large protein devoid of a NLS cannot cross the nuclear membrane in either direction. However, if a NLS is grafted onto this protein, it not only accumulates in the nucleus but also continuously shuttles between the nucleus and the cytoplasm. This movement must occur through the nuclear pore (1, 2), but electron microscopic observations will be necessary for direct confirmation. Accumulation of proteins in the nucleus requires energy whereas their efflux from the nucleus is ATP-independent. It is not clear whether it is the entry into the nucleus that is energy-dependent or whether energy is only necessary for the intranuclear accumulation of the proteins against a gradient of concentration. The export of some ribonucleoproteins has been shown to be energy-dependent (37) and may, thus, occur through a different pathway.

Slow Nucleocytoplasmic Shuttling of the DNA-Binding SV40 Large T Antigen. The experiments described above suggest that all proteins carrying karyophilic signals should be shuttling between the nucleus and the cytoplasm. Such a conclusion is, however, contradictory to some previous observations (11, 12). The most likely explanation for this discrepancy could be the fact that several nuclear proteins form complexes with other intranuclear components (DNA, proteins of the chromatin, etc.) (15) and, thus, only the small fraction of the protein that is unbound is available for the shuttling mechanism. If this hypothesis is true, then in heterokaryons, the transfer of such proteins from one nucleus to another should occur, but at a markedly slower pace when compared to proteins that are not complexed to nuclear components. To test this point, we prepared heterokaryons between simian COS-7 cells containing SV40 large T antigen and mouse L cells devoid of this protein. The transfer of the antigen was practically not detectable at 6 h but was readily observed at 24 h (Fig. 5). A control experiment was performed in heterokaryons composed of L cells expressing nlsSV40-β-galactosidase and human 293 cells. With this protein, accumulation in human nuclei was already maximal at 6 h (Fig. 3). Moreover, administration of sodium azide for 4 h had no effect on the nuclear localization of SV40 large T antigen (data not shown). Such a treatment has been shown to provoke the efflux from the nucleus of nlsSV40-β-galactosidase (see Fig. 3*B*). Thus the karyophilic signal of the large T antigen does impart the ability to shuttle through the nuclear membrane to heterologous proteins. However, in the context of the SV40 large T antigen itself, which is a DNA binding protein, this phenomenon is slow and thus more difficult to observe. Previous negative results (11) involved observation of heterokaryons 4 h after the fusion, a time

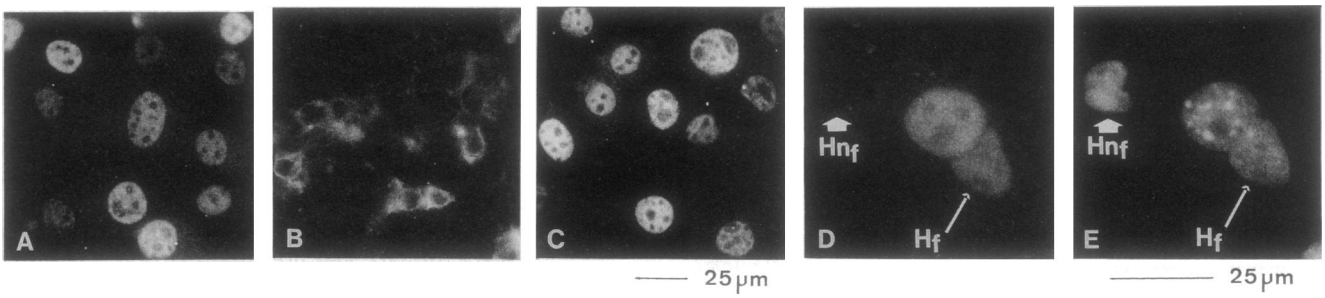

FIG. 3. Nucleocytoplasmic shuttling of nlsSV40-β-galactosidase. (*A–C*) In an L-cell line permanently expressing nlsSV40-β-galactosidase, the β-galactosidase was present in the nucleus (*A*). If the cells were cultured for 4 h in the presence of 2-deoxyglucose and sodium azide (10 mM), β-galactosidase shifted into the cytoplasm (*B*). (The same result was observed whether 10 μM cycloheximide was present or not.) If the cells were again transferred for 4 h into a medium containing glucose and devoid of sodium azide, β-galactosidase was detected in the nucleus (*C*). The same result was observed in COS-7 cells transiently transfected with the same expression vector (data not shown). (*D* and *E*) Mouse L cells expressing nlsSV40-β-galactosidase were fused with 293 human cells and the heterokaryons were observed after 16 h. Cycloheximide was added as described (9, 22). Staining with Hoechst 33258 allowed distinction between human (H) and mouse (M) nuclei (*E*). After the fusion, β-galactosidase (*D*) appeared in the human nucleus included in the heterokaryon (Hf = human, fused). Note the absence of β-galactosidase in the human nucleus not included in the heterokaryons (Hnf = human, nonfused). Identical observations were made 4 h and 6 h after the fusion. Nucleocytoplasmic shuttling was also observed (in the presence of cycloheximide at 10 μg/ml or 100 μg/ml) 4 h after the fusion of mouse L cells with COS-7 cells transiently expressing nlsSV40-β-galactosidase (data not shown).

probably too short to allow the dissociation of a major fraction of the protein from the DNA and its subsequent transfer into the cytoplasm.

These results are in agreement with those of Schmidt-Zachmann *et al.* (16) who demonstrated that deletions of the regions involved in intranuclear binding resulted in a more readily observable shuttling of nuclear proteins. These authors proposed, however, that the nuclear export is a non-mediated passive process. Their hypothesis is in contradiction with the present experiments that suggest that although being non-energy-dependent, the nuclear egress of proteins is NLS-mediated. It is also in contradiction with previous reports (4, 13, 14) showing that a nonnuclear protein microinjected into the nucleus cannot gain access to the cytoplasm. As stressed by Laskey and Dingwall (15), this hypothesis also cannot explain why the nucleoplasmin core is retained in the nucleus after microinjection. Furthermore, the possibility of a free passive efflux of large proteins from the nucleus is difficult to reconcile with all the convergent structural data on the anatomy of the nuclear pore. Aqueous channels of a size limited to 9–10 nm have been observed (for review, see refs. 1 and 2). The size of these channels prevents the free diffusion into the nucleus of proteins >60 kDa (38). It is difficult to understand how such channels could accommodate the unlimited passive diffusion of large proteins in the outward direction.

Our data imply that all proteins bearing a NLS should be able to shuttle between the nucleus and the cytoplasm. Their subcellular localization will, therefore, result from their energy-dependent accumulation in the nucleus but also from their possible interactions with nuclear and/or cytoplasmic components. Examples of such indirect regulations of the localization of nuclear proteins have been described (39, 40). The role for ATP in the shuttling process is not clear. Both nuclear import and export cannot be ascribed to a passive diffusion since they are both NLS-mediated, possibly through interactions with NLS binding proteins. However,

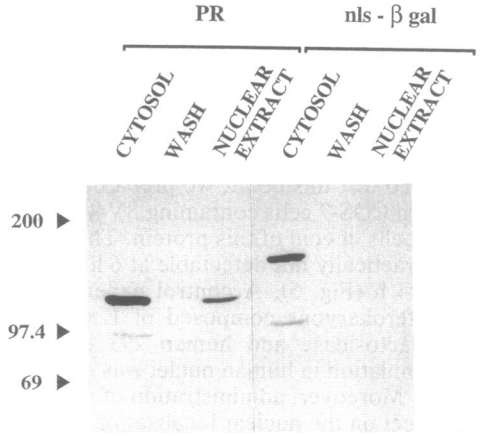

FIG. 4. Nuclear protein nls-β-galactosidase is recovered in the cytosolic fraction of cell homogenates. COS-7 cells were transfected with the expression vector pKSV-rPR encoding the wild-type PR (18) or with nlsSV40-β-galactosidase (nls-β-gal). The cells were homogenized, and cytosol, nuclear wash, and nuclear extract fractions were prepared. These fractions were electrophoresed on a polyacrylamide gel and the proteins were detected by immunoblot analysis. The cytosolic fraction contained 86% of PR and 99% of nls-β-galactosidase. Molecular masses are shown in kilodaltons.

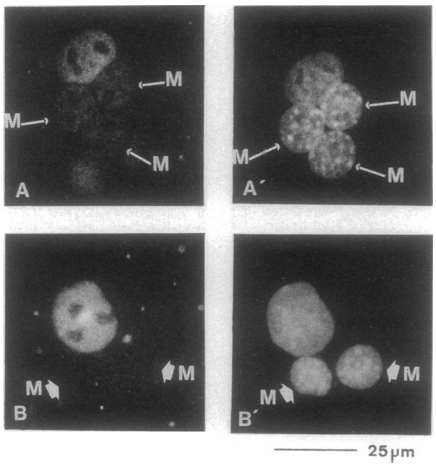

FIG. 5. Slow transfer of SV40 large T antigen from simian to mouse nuclei in heterokaryons. (*A* and *A'*) Simian COS-7 cells were fused with mouse L cells and the heterokaryons were observed 24 h after fusion in the presence of cycloheximide. SV40 large T antigen was present in the nuclei of COS-7 cells. After the fusion, it appeared in the nuclei of mouse cells (M). Unmarked nuclei belong to simian cells. Twenty heterokaryons were observed. Sixteen hours after the fusion, all the mouse nuclei involved in heterokaryons contained the large T antigen. Six hours after the fusion, a single labeled mouse nucleus was observed. (*B* and *B'*) Control experiment in which adjacent cells have not been fused. The thick arrows indicate mouse L-cell nuclei (M) in which the large T antigen is absent. (*A* and *B*) Immunolocalization of SV40 large T antigen. (*A'* and *B'*) Hoechst 33258 fluorescence was used to visualize DNA.

only protein entry or accumulation in the nucleus is energy-dependent. ATP may possibly act as a substrate for protein phosphorylation. Phosphorylation of the protein undergoing shuttling seems unlikely since completely unrelated proteins are involved (SV40 large T antigen and bacterial β-galactosidase in the present report). More likely could be a mechanism involving a phosphorylation–dephosphorylation cycle of the carrier protein(s) with which the karyophilic signals have been shown to interact (1, 2). Such a cycle could be responsible for the asymmetrical accumulation of the proteins in the nucleus.

The shuttling properties imparted by the karyophilic signals may have important consequences not only for the understanding of the biology of the nuclear pore but also for the regulation of nuclear functions. During the shuttling process nuclear proteins and specially those involved in RNA transcription and DNA replication (40) could undergo modifications in the cytoplasm where they could also exert specific biological activities.

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1. Nigg, E. A., Baeuerle, P. A. & Lührmann, R. (1991) *Cell* **66**, 15–22.
2. Davis, L. I. (1992) *Curr. Opin. Cell Biol.* **4**, 424–429.
3. Kalderon, D., Roberts, B. L., Richardson, W. D. & Smith, A. E. (1984) *Cell* **39**, 499–509.
4. Lanford, R. E., Kanda, P. & Kennedy, R. C. (1986) *Cell* **46**, 575–582.
5. Robbins, J., Dilworth, S. M., Laskey, R. A. & Dingwall, C. (1991) *Cell* **64**, 615–623.
6. Ylikomi, T., Bocquel, M. T., Berry, M., Gronemeyer, H. & Chambon, P. (1992) *EMBO J.* **11**, 3681–3694.
7. Borer, R. A., Lehner, C. F., Eppenberger, H. M. & Nigg, E. A. (1989) *Cell* **56**, 379–390.
8. Meier, U. T. & Blobel, G. (1992) *Cell* **70**, 127–138.
9. Guiochon-Mantel, A., Lescop, P., Christin-Maitre, S., Loosfelt, H., Perrot-Applanat, M. & Milgrom, E. (1991) *EMBO J.* **10**, 3851–3859.
10. Dauvois, S., White, R. & Parker, M. G. (1993) *J. Cell Sci.* **106**, 1377–1388.
11. Chandran, U. R. & DeFranco, D. B. (1992) *Mol. Endocrinol.* **6**, 837–844.
12. Madan, A. P. & DeFranco, D. B. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 3588–3592.
13. Mandell, R. B. & Feldherr, C. M. (1990) *J. Cell Biol.* **111**, 1775–1783.
14. Dingwall, C., Sharnick, S. V. & Laskey, R. A. (1982) *Cell* **30**, 449–458.
15. Laskey, R. A. & Dingwall, C. (1993) *Cell* **74**, 585–586.
16. Schmidt-Zachmann, M. S., Dargemont, C., Kühn, L. C. & Nigg, E. A. (1993) *Cell* **74**, 493–504.
17. Guiochon-Mantel, A., Loosfelt, H., Lescop, P., Sar, S., Atger, M., Perrot-Applanat, M. & Milgrom, E. (1989) *Cell* **57**, 1147–1154.
18. Guiochon-Mantel, A., Loosfelt, H., Ragot, T., Bailly, A., Atger, M., Misrahi, M., Perricaudet, M. & Milgrom, E. (1988) *Nature (London)* **336**, 695–698.
19. Lorenzo, F., Jolivet, A., Loosfelt, H., Vu Hai, M. T., Brailly, S., Perrot-Applanat, M. & Milgrom, E. (1988) *Eur. J. Biochem.* **176**, 53–60.
20. Tsuneoka, M. & Mekada, E. (1992) *J. Biol. Chem.* **267**, 9107–9111.
21. Sanes, J. R., Rubenstein, J. L. R. & Nicolas, J. F. (1986) *EMBO J.* **5**, 3133–3142.
22. Powell, L. & Burke, B. (1990) *J. Cell Biol.* **111**, 2225–2234.
23. Madsen, P., Nielsen, S. & Celis, J. E. (1986) *J. Cell Biol.* **103**, 2083–2089.
24. Nardulli, A. M. & Katzenellenbogen, B. S. (1986) *Endocrinology* **119**, 2038–2046.
25. Logeat, F., Pamphile, R., Loosfelt, H., Jolivet, A., Fournier, A. & Milgrom, E. (1985) *Biochemistry* **24**, 1029–1035.
26. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
27. Jensen, E. V., Suzuki, J., Kawashima, T., Stumpf, W. E., Jungblut, S. W. & Desombre, E. R. (1968) *Proc. Natl. Acad. Sci. USA* **59**, 632–636.
28. Gorski, J., Toft, D., Shyamala, G., Smith, D. & Notides, A. (1968) *Rec. Prog. Horm. Res.* **24**, 45–80.
29. King, W. J. & Greene, G. L. (1984) *Nature (London)* **307**, 745–749.
30. Welshons, W. V., Lieberman, M. E. & Gorski, J. (1984) *Nature (London)* **307**, 747–749.
31. Perrot-Applanat, M., Logeat, F., Groyer-Picard, M. T. & Milgrom, E. (1985) *Endocrinology* **116**, 1473–1484.
32. Welshons, W. V., Krummel, B. M. & Gorski, J. (1985) *Endocrinology* **117**, 2140–2147.
33. Paine, P. L., Austerberry, C. F., Desjarlais, L. J. & Horowitz, S. B. (1983) *J. Cell Biol.* **97**, 1240–1242.
34. Evan, G. I. & Hancock, D. C. (1985) *Cell* **43**, 253–261.
35. Lindahl, T. & Barnes, D. E. (1992) *Annu. Rev. Biochem.* **61**, 251–281.
36. Meier, U. T. & Blobel, G. (1990) *J. Cell Biol.* **111**, 2235–2245.
37. Pinol-Roma, S. & Dreyfuss, G. (1992) *Nature (London)* **355**, 730–732.
38. Paine, P. L., Moore, L. C. & Horowitz, S. B. (1975) *Nature (London)* **254**, 109–114.
39. Kambach, C. & Mattaj, I. W. (1992) *J. Cell Biol.* **118**, 11–21.
40. Whiteside, S. T. & Goodbourn, S. (1993) *J. Cell Sci.* **104**, 949–955.