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Bidirectional transport of glucocorticoid receptors across the nuclear envelope

(nuclear import/nuclear export)

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ABSTRACT **We have used transient interspecies heterokaryons to analyze nucleocytoplasmic shuttling of glucocorticoid receptor (GR) and demonstrated that receptors that accumulate within nuclei upon ligand binding are not statically confined to that compartment, but rather have the capacity to reversibly traverse the nuclear envelope. The ability of various GR mutants to shuttle between nuclei of heterokaryons excluded transcriptional activation and DNA binding as prerequisites for nucleocytoplasmic shuttling of GR. However, a constitutively nuclear GR derivative that has fused at its amino terminus the simian virus 40 large tumor antigen nuclear localization signal sequence was unable to efficiently export from nuclei unless ligand-bound. These results uncover an unexpected effect of ligand binding to GR—i.e., the overriding of a dominant negative effect on nuclear export of a heterologous nuclear import signal sequence. Furthermore, they demonstrate that a nuclear import signal sequence can influence nuclear processing pathways that culminate in efficient nuclear export.**

Steroid hormones are important regulators of cellular development and differentiation whose functions are mediated by cognate intracellular receptor proteins. Steroid hormone receptors, as members of a large superfamily of ligand-activated transcription factors, modulate transcription from target genes that possess specific sequence elements that are recognized by corresponding receptor proteins (1). Although ligand binding to all steroid receptor proteins initiates their transformation from a weak to tight DNA binding form, the equilibrium subcellular distribution of unliganded receptors is not identical. For example, unliganded progesterone and estrogen receptors are predominantly nuclear (2, 3), whereas unliganded glucocorticoid receptors (GRs) are usually localized within the cytoplasmic compartment (4, 5). Ligand binding to the GR leads to its rapid and efficient translocation into the nucleus ($t_{1/2} \approx 5$–10 min) (4, 5). The GR contains two distinct nuclear localization signals (NLSs), NL1 and NL2, that can function when fused to heterologous proteins to target them to the nucleus in a hormone-independent and -dependent manner, respectively (5). NL1 is localized within a 27-amino acid segment that is adjacent to the DNA binding domain, whereas NL2 is located within the carboxyl-terminal hormone binding domain. The ability of NL2 to mask NL1 function (5) is most likely responsible for the predominant cytoplasmic localization of unliganded GR observed in some cells.

Despite extensive characterization of protein import into nuclei, mechanisms of protein export from nuclei remain enigmatic. For large karyophilic proteins such as GR, nuclear import and export are most likely facilitated processes and occur through the nuclear pore. Only a subset of cellular proteins that are imported into nuclei are capable of being exported to the cytoplasm. For example, major nucleolar proteins nucleolin and the B23/No38 antigen (6) and the small nuclear ribonucleoprotein component La antigen (7) have been shown by direct methods to shuttle between nuclear and cytoplasmic compartments. The apparent redistribution of GR from the nucleus to the cytoplasm under conditions of hormone withdrawal implies that it too has the capacity to bidirectionally traverse the nuclear envelope (8, 9). Thus, it seems likely that the subcellular localization of GR reflects a dynamic process with factors such as hormone binding influencing the relative activity of nuclear import or putative export signal sequences contained within the receptor. We have used transient heterokaryons (6, 7) to directly analyze bidirectional transport (i.e., nucleocytoplasmic shuttling) of GR across the nuclear envelope. Examination of the nucleocytoplasmic shuttling properties of various GR mutants has provided information concerning the prerequisites of receptor nuclear export and uncovered an inhibitory effect of a linked heterologous NLS on GR nuclear export.

MATERIALS AND METHODS

Cell Lines. GrH2 (10), a rat hepatoma cell line, and COS-1 monkey kidney fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM; GIBCO–Bethesda Research Laboratories) supplemented with 10% fetal bovine serum (Irvine Scientific). A GR-negative mouse NIH 3T3 derivative cell line (obtained from J. Pipas, University of Pittsburgh) was maintained in DMEM supplemented with 5% defined calf serum (HyClone).

Inhibition of Protein Synthesis by Cycloheximide. GrH2 cells were cultured in medium containing 200 μCi of [^{35}S]methionine (9.67 mCi/ml; 1 Ci = 37 GBq; ICN) in the presence or absence of 200 μg of cycloheximide (Sigma) per ml for 1, 2, 4, and 8 hr. After appropriate time intervals, cells were harvested and whole cell extracts were prepared. GR was immunoabsorbed to protein A-Sepharose (Pharmacia) with the BuGR2 monoclonal antibody (11) as described (12) and electrophoresed through 7.5% SDS/polyacrylamide gels (SDS/PAGE). Electrophoresed proteins were analyzed by autoradiography.

Measurement of Half-Life of GR in GrH2 Cells. Pulse–chase labeling was used to determine the half-life of GR in GrH2 cells. Cells were pulse-labeled for 30 min in medium containing 200 μCi of [^{35}S]methionine. After washing, medium containing excess unlabeled methionine (1 mg/ml) was added to the cells and incubation was continued. At different times after addition of unlabeled methionine, cells were harvested and whole cell extracts were prepared. GR was immunoabsorbed to protein A-Sepharose using the BuGr2 monoclonal

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Abbreviations: GR, glucocorticoid receptor; SV40, simian virus 40; TAg, large tumor antigen; NLS, nuclear localization signal; β-Gal, β-galactosidase; TRITC, tetramethylrhodamine B isothiocyanate; Dex, dexamethasone; IIF, indirect immunofluorescence.

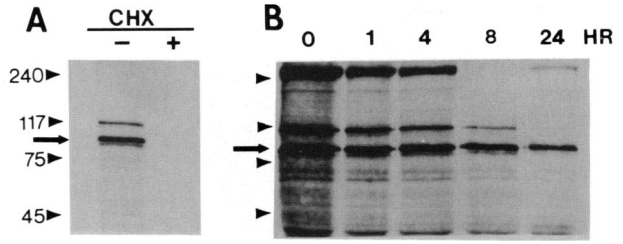

FIG. 1. Analysis of GR stability in GrH2 cells. (*A*) GrH2 cells were labeled with [^{35}S]methionine with or without 200 μg of cycloheximide (CHX) per ml. GR was isolated and visualized over a period of 8 hr by immunoprecipitation and SDS/PAGE. Autoradiograph shows GR (arrow) isolated at 4 hr after labeling without (−) cycloheximide and with (+) cycloheximide. Western blot analysis was used to confirm the identity of GR (data not shown). (*B*) For determination of half-life of GR, GrH2 cells were labeled with [^{35}S]methionine for 30 min. Medium containing unlabeled methionine was added to cells and the half-life of GR was determined following immunoprecipitation of GR (12) and quantitation of GR (arrow) levels by scanning densitometry of autoradiographs. Molecular weight markers are indicated by arrowheads. Western blot analysis was used to confirm the identity of GR (data not shown).

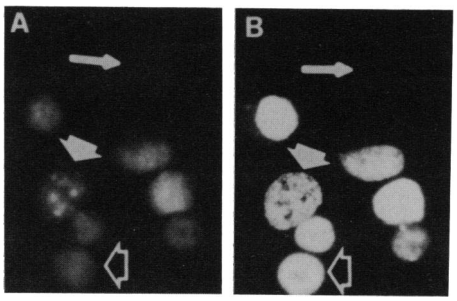

FIG. 2. Translocation of endogenous GR from GrH2 cell nuclei to NIH 3T3 nuclei in transient heterokaryons. GrH2 cells treated with 1 μM Dex for 1 hr were fused to NIH 3T3 cells using 50% PEG. Immediately after fusion, cycloheximide and 1 μM Dex were added to the cells. Four hours after fusion cells were fixed and stained with Hoechst dye (*A*) to distinguish GrH2 nuclei (open arrow, uniform staining) from NIH 3T3 nuclei (solid arrow, punctate staining). (*B*) Same field as in *A*, stained with the BuGR2 monoclonal antibody to GR (11) and a fluorescein-conjugated secondary antibody. Shuttling of GR from GrH2 to NIH 3T3 nuclei can be visualized by detection of GR in NIH 3T3 nuclei of heterokaryons. The GR-negative NIH 3T3 nucleus in an unfused cell shows no staining above background (long arrow).

antibody and analyzed by SDS/PAGE. GR was visualized by autoradiography. The relative amount of GR protein was determined by laser scanning densitometry of autoradiographs (LKB Ultoscan XL).

Formation of Interspecies Heterokaryons. Cells were seeded onto single coverslips and allowed to attach in DMEM containing 10% fetal bovine serum. COS-1 cells were transiently transfected with a wild-type GR expression plasmid, VARO (5), or the following mutants, where appropriate: GR SV.4C (13), the amino-terminal deletion mutant VA407C in which 407 amino acids have been removed (14), the carboxyl-terminal deletion mutant VAN556 in which 239 amino acids have been removed (14), or the DNA binding mutant VARO C500Y in which a cysteine at amino acid position 500 has been replaced by a tyrosine (15) before seeding the second cell line. Following cell attachment, phenol red-free DMEM containing 10% charcoal-stripped serum (Sigma) was added to cultures and incubation was continued for 16–18 hr. When appropriate, 1 μM dexamethasone (Dex) (Sigma) or 1 μM RU486 (Roussel-Uclaf) was added to cells 1 hr before fusion. Fusions were carried out by inverting the coverslips on prewarmed 50% PEG 6000 (Sigma) in calcium/magnesium-free Hanks' solution (6) for 2 min. The coverslips were then washed extensively in Hanks' solution and incubated for 4 hr in the presence of 200 μg of cycloheximide per ml and, where appropriate, 1 μM Dex or 1 μM RU486.

Immunofluorescence Assays. Indirect immunofluorescence assays were carried out as described (4). Briefly, after three washes with ice-cold phosphate-buffered saline (PBS), cells were fixed with cold (−20°C) methanol for 5 min. Fixed cells were overlaid with the BuGr2 monoclonal antibody and then

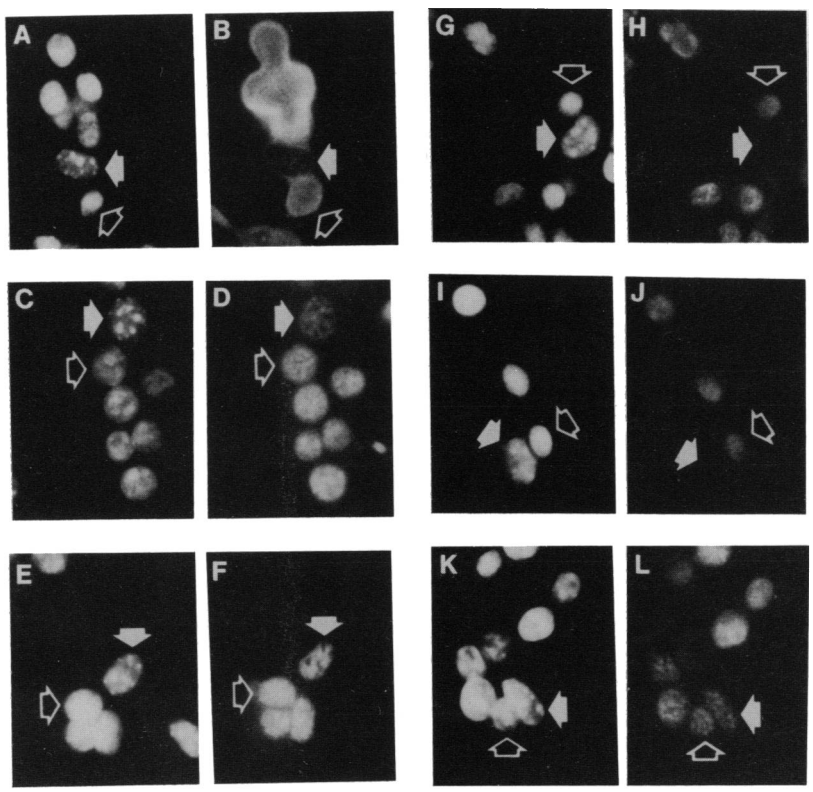

FIG. 3. Establishment of internuclear migration of GR in transient heterokaryons. GrH2/NIH 3T3 cell heterokaryons were generated either without the addition of Dex (*A–F*), to localize GR to the cytoplasm, or after treatment with Dex for 1 hr (*G–L*), to localize GR to GrH2 nuclei. After fusion, Dex and cycloheximide were added to all cells. Samples were fixed at 0 hr (*A* and *B* and *G* and *H*), 10 min (*C* and *D* and *I* and *J*), and 1 hr (*E* and *F* and *K* and *L*) after fusion, fixed, and stained for GR with the BuGR2 monoclonal antibody (11) and for DNA with Hoechst dye. Panels on the left show Hoechst dye staining to differentiate NIH 3T3 nuclei (solid arrow, punctate staining) from GrH2 nuclei (open arrow, uniform staining). Panels on the right show the same cells stained for GR. GR is detected in NIH 3T3 nuclei 10 min following fusion and initial addition of Dex (*C* and *D*), whereas when GR was localized to GrH2 nuclei prior to fusion, GR was not detected within NIH 3T3 nuclei until 1 hr after fusion (*K* and *L*).

with a fluorescein-conjugated anti-mouse antibody (Boehringer Mannheim) to visualize GR. For β-galactosidase (β-Gal) staining, fixed cells were treated with an anti-β-Gal polyclonal antibody (Sigma) and a secondary antibody coupled to tetramethylrhodamine B isothiocyanate (TRITC; Sigma). With the secondary antibody, cells were also stained with 0.5 μg of Hoechst dye no. 33258 per ml (Calbiochem) to distinguish nuclei. Stained cells were observed by fluorescence microscopy using a Nikon Labophot microscope fitted with appropriate combinations of excitation and barrier filters.

RESULTS

Nucleocytoplasmic Shuttling of GR in GrH2 Cells. Interspecies transient heterokaryons have provided one of the few experimental systems for analysis of protein nuclear export (6, 7). We and others (16, 17) have already demonstrated their usefulness in examining progesterone receptor nuclear export, and we describe in this report the application of this system to the study of GR nucleocytoplasmic shuttling. Interspecies heterokaryons were generated between GrH2, a rat hepatoma cell line that contains elevated levels of GR (10), and a mouse NIH 3T3 derivative that lacks immunodetectable GR. Since unliganded GRs reside predominantly in the cytoplasm of GrH2 cells (e.g., see Fig. 3), the synthetic glucocorticoid Dex was added to cells 1 hr prior to cell fusion to localize GR within the nucleus. Following cell fusion with 50% PEG (6), cycloheximide was added to inhibit *de novo* protein synthesis. As shown in Fig. 1*A*, 200 μg of cycloheximide per ml effectively inhibited GR synthesis in GrH2 cells. Results from pulse–chase experiments demonstrate that the half-life of GR in Dex-treated GrH2 cells is ≈9 hr (Fig. 1*B*). These results establish the validity of our cell fusion paradigm for monitoring internuclear migration of GR and ensure that any GR detected within NIH 3T3 nuclei most likely derives from receptors that preexisted within GrH2 nuclei, and not from *de novo* synthesized receptors. Fused cells were fixed after a 4-hr incubation in medium that contained Dex, and GRs were detected by indirect immunofluorescence (IIF) (4). The nuclei of the two cell types were distinguished by their differential staining with Hoechst dye (16) (Fig. 2*A*). As shown in Fig. 2*B*, GRs were detected within NIH 3T3 nuclei of GrH2/NIH 3T3 heterokaryons, demonstrating that Dex-bound GRs derived from GrH2 nuclei were capable of being exported from GrH2 nuclei and shuttling to available NIH 3T3 nuclei within a multinucleate heterokaryon. Identical results were obtained when COS-1 cells transiently transfected with a rat GR expression vector were fused to NIH 3T3 cells (data not shown).

To eliminate any possibility that GR within NIH 3T3 nuclei of the heterokaryons arose from nuclear import of residual cytoplasmic GR undetectable by IIF, we took advantage of the apparent difference in rate of nuclear import versus export of GR. Hormone-dependent nuclear translocation of GR has been shown to be very rapid, with a $t_{1/2}$ of 5–10 min (5). However, in hormone withdrawal experiments, redistribution of GR from the nucleus to the cytoplasm appeared to take considerably longer (4). We therefore generated GrH2/NIH 3T3 heterokaryons in the absence of hormone and added Dex following cell fusion to initiate nuclear import of cytoplasmic GR in multinucleate heterokaryons. As transport of GR from the cytoplasm to the nucleus is rapid (5), NIH 3T3 nuclei of heterokaryons exhibited maximum GR staining within 10 min following hormone addition (Fig. 3 *C* and *D*). However, in heterokaryons where GR was localized to the nucleus prior to fusion by the addition of Dex, GR was not detected within NIH 3T3 nuclei of heterokaryons until 1 hr following cell fusion (Fig. 3 *K* and *L*). These results firmly establish that GRs in NIH 3T3 nuclei of heterokaryons arise predominantly from the export of preexistent receptors from GrH2 nuclei.

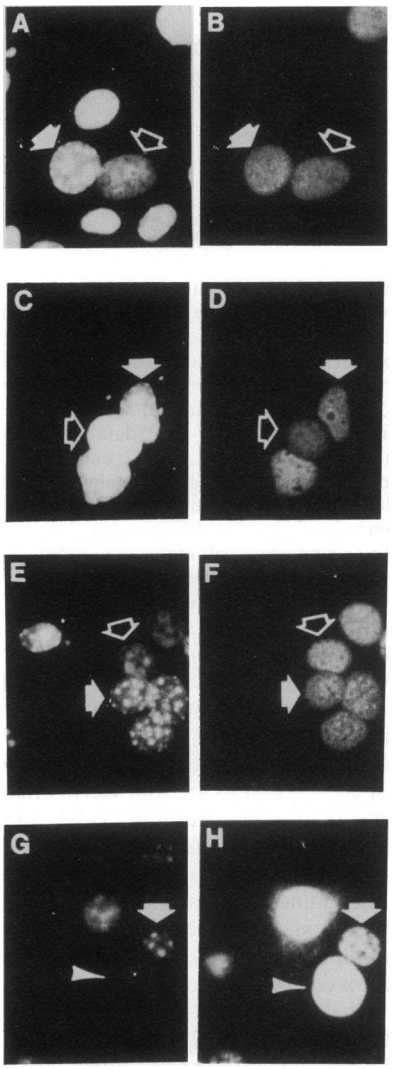

FIG. 4. Nucleocytoplasmic shuttling of GR mutants. COS-1 cells transfected with GR expression plasmids VAN556 (hormone binding deficient; ref. 14) (*A* and *B*), VA407C (transactivation deficient; ref. 14) (*C* and *D*) and VARO C500Y (deficient in DNA binding; ref. 15) (*E* and *F*) were fused to NIH 3T3 cells and fixed 4 hr after fusion. (*G* and *H*) GrH2 cells were treated with RU486 for 1 hr before fusion with NIH 3T3 cells to localize GR within nuclei. Heterokaryons were fixed 4 hr after fusion. Nuclei were distinguished with Hoechst dye (solid arrow, NIH 3T3; open arrow, COS-1; arrowhead, GrH2) and GR was detected with the BuGR2 monoclonal antibody (11) and a secondary antibody coupled to fluorescein. Panels on the left show Hoechst dye staining and panels on the right are identical fields stained for GR.

Prerequisites of GR Shuttling. Like all members of the steroid/thyroid hormone receptor superfamily, GR is comprised of discrete separable domains that function in transactivation, DNA binding, hormone binding, and nuclear localization (5, 14, 18). In an attempt to define activities of GR that may be important for its nucleocytoplasmic shuttling, we tested the ability of various GR mutants to shuttle between nuclei in interspecies heterokaryons. COS-1 cells were transiently transfected with carboxyl- and amino-terminal deletion mutants, VAN556 (14) and VA407C (14), respectively, and fused to NIH 3T3 cells. Since VAN556, which lacks a hormone binding domain, is constitutively nuclear, transient heterokaryons were generated and analyzed in the absence of hormone. Hormone was added 1 hr prior to cell fusion for

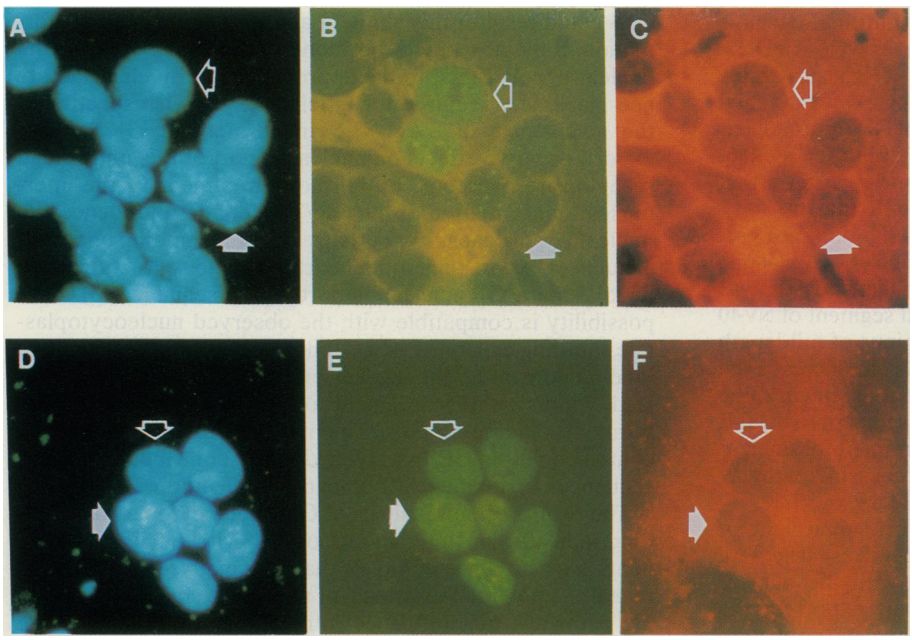

FIG. 5. Hormone-dependent nucleocytoplasmic shuttling of a GR/TAg chimera. COS-1 cells transiently transfected with the SV.4C GR expression plasmid (13) were fused to NIH 3T3 cells either in the absence (*A–C*) or presence (*D–F*) of Dex. Cells were fixed and stained with Hoechst dye (panels on left), to distinguish COS-1 nuclei (open arrow, uniform staining) from NIH 3T3 nuclei (solid arrow, punctate staining) and stained for GR as indicated earlier. GR staining was not detected in NIH 3T3 nuclei of transient heterokaryons in the absence of hormone (*B*), whereas GR staining was detected in NIH 3T3 nuclei when Dex was added (*E*). To distinguish fused cells from cells in close proximity, heterokaryons were costained with a β-Gal antibody and a secondary antibody coupled to TRITC. The shared cytoplasm of the heterokaryons can be visualized in *C* and *F*. A few cells exhibit intense TRITC β-Gal staining that escapes filter blocking and is detected in pale red in fluorescein isothiocyanate images.

VA407C transfected COS-1 cells to import receptors into nuclei. As shown in Fig. 4 *A–D*, both mutant GRs were detected within NIH 3T3 nuclei of heterokaryons, indicating that neither the amino-terminal 407 nor the carboxyl-terminal 239 amino acids of GR are absolutely required for its nuclear export and subsequent nuclear reentry. Furthermore, these results demonstrate that the hormone-dependent nuclear import signal, NL2, can be deleted without any obvious impact upon receptor shuttling.

To ascertain the role of DNA binding in nucleocytoplasmic shuttling of GR, COS-1 cells transiently transfected with the GR DNA binding point mutant VARO C500Y (15) were fused to NIH 3T3 cells. This mutant receptor was capable of migrating from COS-1 nuclei to NIH 3T3 nuclei in heterokaryons, demonstrating that DNA binding is also not essential for GR nucleocytoplasmic shuttling (Fig. 4*F*). Finally, to determine whether nucleocytoplasmic shuttling of GR required its functioning as a transcriptional modulator, we used the hormone antagonist RU486 to import GR into nuclei prior to performing fusions with NIH 3T3 cells. RU486-bound wild-type GRs, although capable of translocating to the nucleus, are incapable of transcriptionally activating GR-responsive genes (19). As shown in Fig. 4 *G* and *H*, GR was detected within NIH 3T3 nuclei of heterokaryons containing either RU486-treated GrH2 cells or COS-1 cells transfected with a wild-type GR expression plasmid (data not shown). Thus, nucleocytoplasmic shuttling of GR can occur independent of its participation in transcriptional modulatory events. The fact that we could not detect any partial agonist activity of RU486 in GrH2 cells and transfected COS-1 cells (data not shown) supports this conclusion.

Hormone-Dependent Nucleocytoplasmic Shuttling of a GR/Large Tumor Antigen (TAg) Chimera. Recent studies have demonstrated efficient nucleocytoplasmic shuttling of unliganded and hormone-bound progesterone receptor in transient heterokaryons (16, 17). To determine whether unliganded full-length GR has the capacity to shuttle between nuclei of heterokaryons, we utilized a GR chimera, termed SV.4C (13), that possesses at its amino terminus a 26-amino acid segment of simian virus 40 (SV40) TAg that includes its NLS. The TAg NLS in this chimera is presumedly functional since it overrides the dominant effect of the hormone-dependent GR NLS (i.e., NL2) leading to constitutive nuclear localization of this chimera (13). COS-1 cells were transiently transfected with an SV.4C expression plasmid and nucleocytoplasmic shuttling of this GR/TAg chimera was monitored in COS-1/NIH 3T3 heterokaryons in the presence and absence of hormone. In the presence of Dex, SV.4C GR was detected within NIH 3T3 nuclei of heterokaryons (Fig. 5*E*) demonstrating the nucleocytoplasmic shuttling capacity of this chimeric receptor. In contrast, unliganded SV.4C GR did not appear to efficiently export from COS-1 nuclei, as this chimeric receptor was not detected within NIH 3T3 nuclei of heterokaryons not treated with hormone (Fig. 5*B*). In the case of this negative result it becomes essential to distinguish fused cells from unfused cells that are in close proximity. Therefore, we used an NIH 3T3 cell line that was stably transfected with a β-Gal expression vector as the recipient cell in the generation of heterokaryons. Cytoplasmic β-Gal protein staining within the shared cytoplasm of heterokaryons provides an unequivocal demonstration of cell fusion. The detection of β-Gal costaining (Fig. 5*C*) in shared cytoplasm of heterokaryons generated between SV.4C GR transfected COS-1 cells and NIH 3T3 cells in the absence of hormone confirms the inefficient nuclear export of the unliganded GR/TAg chimera (Fig. 5*B*).

DISCUSSION

We have used transient interspecies heterokaryons to analyze nucleocytoplasmic shuttling of GR and demonstrated that receptors that accumulate within nuclei upon ligand binding are not statically confined to that compartment but, rather, have the capacity to reversibly traverse the nuclear envelope. Thus, the accumulation of GRs within any given subcellular compartment is most likely governed by whether nuclear import or export is rate limiting in this dynamic translocation process. To better define activities of GR that

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          119                                  144
           |                                    |
TAg:       DSQHSTPPKKKRKVEDPKDFPSELLS

          497                                  524
           |                                    |
rGR:       YRKCLQAGMNLEARKTKKKIKGIQQATA
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FIG. 6. NLSs within the rat GR (rGR) and SV40 TAg: amino acid sequence (in single-letter code) from residues 119–144 of the SV40 TAg and residues 497–524 of the rGR which includes their NLSs. Basic amino acid residues, lysine (K) and arginine (R), are indicated in open letters.

may be influencing its nucleocytoplasmic shuttling, we have utilized a carboxyl-terminal deletion mutant as well as a DNA binding point mutant in our shuttling assays. To ascertain the role of transactivation of GR in shuttling, we examined whether antagonist-bound GR was capable of internuclear migration in transient heterokaryons. The ability of RU486-bound GR and various GR mutants to shuttle suggests that none of the well-established functions of GR that we have tested, including DNA binding and transactivation, are prerequisites for receptor nuclear export and nucleocytoplasmic shuttling. However, nuclear export of unliganded GR was impaired by the addition of a 26-amino acid segment of SV40 TAg containing its NLS to the amino terminus of full-length receptor. The addition of extraneous NLSs can increase the rate and efficiency of nuclear import (20), but in our case, the net result of adding a TAg NLS-containing segment to the GR is to bypass the hormone-dependent requirement for nuclear import (5) and interfere with a nuclear processing pathway that normally targets GR for efficient nuclear export.

What is the mechanism responsible for impairment of unliganded GR nuclear export by TAg sequences? The TAg NLS, through inappropriate interactions with the nuclear pore, could exert a direct effect to impair export of the unliganded GR/TAg chimera through the nuclear pore complex. This suggests that nuclear export and import machineries may share some common components. The fact that a monoclonal antibody directed against the nuclear pore complex affects macromolecular import into and export from the nucleus provides strong support for this notion (21). However, it must be emphasized that TAg is involved in a number of nuclear functions, including activation and repression of transcription and DNA replication (22), and is associated with a number of nuclear proteins, including the anti-oncogene proteins Rb and p53 (23). The involvement of TAg in nuclear functions not attributed to steroid receptors, such as DNA replication, might target it to specific compartments of the nucleus that are not necessarily accessible to the nuclear export machinery. This idea is supported by recent results from our laboratory that showed that differential nuclear export capacities of intact TAg and the progesterone receptor in COS-1 cells were correlated with their distinct nuclear compartmentalization (16).

Our current results are striking in that a 26-amino acid segment of TAg containing its NLS was all that was required to impair nuclear export of unliganded GR. Since related NLSs of GR and TAg presumedly interact with common components of the nuclear import machinery, their interactions with internuclear components might be responsible for their diverse effects on nuclear export. A comparison of the sequences immediately surrounding the GR and TAg NLSs (Fig. 6) reveals little conservation outside of the constellation of basic amino acids that have been shown to be absolutely essential for nuclear import activity (24). The TAg segment included with the SV.4C GR derivative possesses a potential *cdc2* phosphorylation site (Thr-124) whose phosphorylation state has been shown to influence TAg DNA replication activity (25) and nuclear import (26). Although it has not been demonstrated whether GR nuclear import efficiency or kinetics is affected by its phosphorylation state, hyperphosphorylation of GR at specific sites is associated with its inefficient nucleocytoplasmic shuttling (8). The analysis of site-directed mutations within the GR and the 26-amino acid segment of TAg should establish whether phosphorylation directly influences export of intact GR or chimeras containing TAg sequences.

Since the functioning of nuclear import signals in yeast and mammalian cells can be context dependent (27, 28), GR may contain a nuclear export signal whose activity may be analogously masked in the unliganded nuclear GR/TAg chimera. This could result if this chimera assumes an unusual conformation in the absence of hormone that does not expose the nuclear export signal to the appropriate internuclear factors and machinery required for efficient export. Irrespective of the mechanism of TAg NLS impairment of GR nuclear export, negative effects on nuclear export of this unliganded receptor are overcome by hormone treatment. If the internuclear conformation of the GR/TAg chimera influences its nuclear export capacity, perhaps hormone binding uncovers a nuclear export signal sequence analogous to its role in uncovering a GR nuclear import signal sequence (5). This possibility is compatible with the observed nucleocytoplasmic shuttling capability of a GR deleted of its entire hormone binding domain as the nuclear export signal could be localized within another segment of the receptor. Likewise, hormone binding could uncover a nuclear targeting signal contained within GR that overrides any contribution of TAg sequences to subnuclear compartmentalization.

The results of our analysis of GR nucleocytoplasmic shuttling indicate that interactions of GR with nuclear components may be more complex than originally envisaged and reveal the potential existence of alternative nuclear processing pathways that influence intracellular trafficking of the receptor. We expect that future studies in this system will not only elucidate mechanisms that regulate nucleocytoplasmic shuttling of GR but may also provide insights into an enigmatic intracellular trafficking pathway—i.e., nuclear export.

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