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ATP-Dependent Release of Glucocorticoid Receptors from the Nuclear Matrix

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Glucocorticoid receptors (GRs) have the capacity to shuttle between the nuclear and cytoplasmic compartments, sharing that trait with other steroid receptors and unrelated nuclear proteins of diverse function. Although nuclear import of steroid receptors, like that of nearly all other karyophilic proteins examined to date, requires ATP, there appear to be different energetic requirements for export of proteins, including steroid receptors, from nuclei. In an attempt to reveal which steps, if any, in the nuclear export pathway utilized by steroid receptors require ATP, we have used indirect immunofluorescence to visualize GRs within cells subjected to a reversible ATP depletion. Under conditions which lead to >95% depletion of cellular ATP levels within 90 min, GRs remain localized within nuclei and do not efflux into the cytoplasm. Under analogous conditions of ATP depletion, transfected progesterone receptors are also retained within nuclei. Importantly, GRs which accumulate within nuclei of ATP-depleted cells are distinguished from nuclear receptors in metabolically active cells by their resistance to in situ extraction with a hypotonic, detergent-containing buffer. GRs in ATP-depleted cells are not permanently trapped in this nuclear compartment, as nuclear receptors rapidly regain their capacity to be extracted upon restoration of cellular ATP, even in the absence of de novo protein synthesis. More extensive extraction of cells with high salt and detergent, coupled with DNase I digestion, established that a significant fraction of GRs in ATP-depleted cells are associated with an RNA-containing nuclear matrix. Quantitative Western blot (immunoblot) analysis confirmed the dramatic increase in GR binding to the nuclear matrix of ATP-depleted cells, while confocal microscopy revealed that GRs are bound to the matrix throughout all planes of the nucleus. ATP depletion does not lead to wholesale collapse of nuclear proteins onto the matrix, as the interaction of a subpopulation of simian virus 40 large tumor antigen with the nuclear matrix is not quantitatively altered in ATP-depleted Cos-1 cells. Nuclear GRs which are not bound to the nuclear matrix of metabolically active cells (i.e., a DNA-binding domain deletion mutant and a β-galactosidase chimera possessing the GR nuclear localization signal sequence) are not recruited to the matrix upon depletion of cellular ATP. Thus, it appears that ATP depletion does not expose the GR to nuclear matrix interactions which are not normally encountered in cells but merely alters the dynamics of such interactions. The dynamic association of steroid receptors with the nuclear matrix may provide a mechanism which is utilized by these regulable transcription factors to facilitate their efficient scanning of the genome.

In recent years, there has been an increased appreciation for the extent to which distinct DNA, RNA, and protein components are organized within the nucleus (68). Much of the framework responsible for maintaining nuclear organization is provided for by the nuclear matrix, which is defined as that compartment of the nucleus which remains following DNase and detergent treatment and following high-salt extraction of soluble proteins and chromatin (5, 21, 56). An interconnected meshwork of filaments that localize both beneath the nuclear membrane and within the interior of the nucleus is the major structural component of the matrix (33). In addition to abundant and minor protein components (23), heterogeneous nuclear RNA is associated with the matrix and appears to have an important role in maintaining structural integrity of the matrix (22). It has become increasingly apparent that macromolecular components involved in DNA replication (37), transcription (40, 78), and RNA splicing (81, 87) may be confined to specific, functional domains of the nucleus via their interaction with the nuclear matrix (68).

Many active transcription units are associated with the nuclear matrix (13, 39, 62) and predominantly utilize short (~200-bp), generally A+T-rich segments of DNA termed matrix attachment regions (MARs) for anchoring to the matrix (27). The importance of MARs in tissue- and cell-type-specific gene expression has been established in model transfection studies and in transgenic animals (71, 82). Although specific MAR-binding proteins have been identified (18, 77), their role in mediating the tissue- and cell-type-specific binding of active genes to the nuclear matrix is not understood. A number of transcription factors have been found to be associated with the nuclear matrix (6, 73, 76) and appear to differentially partition between the matrix and an extractable compartment of the nucleus, depending on such variables as tissue type and growth conditions (70). Therefore, it seems likely that specific mechanisms exist to regulate the exchange of transcription factors between the nuclear matrix and soluble compartments of the nucleus.

The availability of high-specific activity radiolabeled steroids made possible the identification of the first transcription factors bound to the nuclear matrix, i.e., steroid receptors (3). As reviewed by Barrack (2), the binding of steroid receptors to the nuclear matrix appears to be hormone dependent and involves saturable, high-affinity interactions. The centrally located DNA-binding domain (DBD) and carboxy-terminal ligand-binding domain (LBD) are required for nuclear matrix binding of the androgen receptor and glucocorticoid receptor (GR), although the relative contributions of the DBD and LBD to matrix

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binding differ between these two highly related proteins (74). The relative proportions of steroid receptors, particularly the sex steroid receptors (2), that are bound to the nuclear matrix vary among different target tissues. A 10-kDa protein isolated from chick oviduct nuclear matrix binds with high affinity and specificity to the progesterone receptor (PR) (66), suggesting that specific acceptor proteins may be responsible for cell type differences in steroid receptor binding to the matrix. Despite this long-standing recognition of nuclear matrix binding of steroid receptors, the mechanisms which serve to regulate receptor binding to the matrix have yet to be established.

Much has been learned about the subcellular trafficking of steroid receptors through studies of the GR, which in many cells undergoes hormone-dependent nuclear import (10, 12, 58, 60, 80). GRs, as well as other steroid receptors, also have the capacity to export from nuclei (11, 15, 32, 45), sharing that property with a number of shuttling nuclear proteins (8, 47, 51, 59, 79). While there is little disagreement concerning the energy dependence of nuclear protein import (48, 55, 61), there are examples of both energy-dependent (4, 43, 51) and apparent energy-independent nuclear export (32, 54). This disparity also appears to apply to closely related members of the steroid receptor superfamily. Thus, while PRs apparently have the capacity to efflux from the nucleus under some conditions of ATP depletion (32), GRs that import into nuclei appear to remain within that compartment upon ATP depletion (38, 49). Mendel et al. have defined the nuclear GR that is retained within nuclei of ATP-depleted cells as a null receptor, as it also lacks hormone-binding activity (49). Even if multiple pathways of nuclear export exist and are distinguished by their energy dependence, it is unclear why structurally related proteins such as PR and GR might utilize such fundamentally distinct pathways for nuclear export.

The studies described within this report were initially designed to assess the energy dependence of steroid receptor nuclear export. In the course of establishing the apparent energy dependence of both GR and PR nuclear export, we have uncovered an ATP-dependent nuclear event, i.e., release from the nuclear matrix, which is an obligatory prerequisite for nuclear export. Furthermore, our results suggest that steroid receptor association with the nuclear matrix is a dynamic process that could utilize the energy derived from ATP hydrolysis to facilitate efficient scanning of the genome.

MATERIALS AND METHODS

Cells and cell culture. GrH2 cells, a rat hepatoma cell line (36), CHO fibroblasts, and Cos-1 monkey kidney fibroblasts were maintained in Dulbecco's modified essential medium (DMEM; GIBCO-Bethesda Research Laboratories [GIBCO-BRL], Grand Island, N.Y.) supplemented with 10% fetal bovine serum (Irvine Scientific, Santa Ana, Calif.). WCL2 cells, a CHO cell derivative which express ~100,000 copies of stably transfected mouse GR (35), were maintained in DMEM with 10% iron-supplemented calf serum (Irvine Scientific), 40 mg of proline (Sigma Chemical Company, St. Louis, Mo.) per liter, and 3 μM methotrexate (Sigma). Where indicated, cells were treated with 1 μM dexamethasone (Dex; Sigma). Transient transfections of CHO cells with a chicken PR expression plasmid were performed as described previously (11). CHO cell variants stably expressing GR mutants or chimeras were generated upon cotransfection with GR expression plasmids and a plasmid that encodes the bacterial neomycin resistance gene (67). Stable transfectants were obtained from transfected cell cultures following selection in the presence of 400 μg of G418 (GIBCO-BRL) per ml. The following rat GR expression plasmids were used to generate stable transfectants: the carboxy-terminal deletion mutant VAN556, in which 239 amino acids have been removed (29), the amino-terminal deletion mutant VA407C, in which 407 amino acids have been removed (29), VAN-D, in which 270 amino acids have been removed from the carboxy terminus and 38 amino acids have been removed from the second zinc finger within the DBD (i.e., amino acids 461 to 499), and NL1βgal, in which 27 amino acids (i.e., amino acids 497 to 524) comprising the rat GR constitutive nuclear localization signal sequence (NLS) have been fused to the bacterial β-galactosidase (β-Gal) gene (58).

Indirect immunofluorescence. BuGR2 (24), a mouse monoclonal antibody that recognizes a segment just upstream of the mouse GR DBD (63), was used in most indirect immunofluorescence analysis to detect GR in methanol-fixed cells (60). In some instances, a commercially available anti-GR rabbit polyclonal antibody (Affinity BioReagents, Inc., Neshanic, N.J.) was used. Chicken PR was detected by indirect immunofluorescence with the mouse monoclonal antibody PR22 (72), simian virus 40 large tumor antigen (SV40 TAg) was detected with the hamster polyclonal antibody HAT-1 (44), and the nuclear matrix protein (NuMA) was detected with the NuMA mouse monoclonal antibody Ab-1 (Oncogene Science Inc., Uniondale, N.Y.). In some cases, fixed cells were stained with 4,6-diamidino-2-phenylidole (DAPI; Sigma) to visualize DNA.

Depletion of cellular ATP. WCL2 cells were depleted of ATP by culturing for the indicated lengths of time in glucose-free DMEM (GIBCO-BRL) plus 6 mM 2-deoxyglucose (Sigma), 10 mM sodium azide (Sigma), 40 mg of proline per liter, 3 μM methotrexate, and 10% iron-supplemented calf serum. In experiments in which ATP was restored to WCL2 cells, ATP-depleting medium was replaced by glucose-containing DMEM with serum and other supplements (see above) in the presence of 100 μg of cycloheximide (Sigma) per ml to inhibit de novo protein synthesis (45). ATP was depleted from GrH2, Cos-1, and CHO cells by using glucose-free DMEM containing 6 mM 2-deoxyglucose and 10% fetal bovine serum. ATP levels were measured in whole cell extracts, as described previously (38), using an ATP Bioluminescence CLS kit (Boehringer Mannheim Corp., Indianapolis, Ind.)

In situ nuclear extractions. Cells grown on glass coverslips (22 by 22 mm), either under aerobic conditions or depleted of ATP, were extracted in situ by a previously described method (53). Briefly, cells were subjected to hypotonic shock by a brief immersion in water and then treated for 5 min at 0°C with hypotonic buffer (10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.9], 10 mM potassium chloride, 1.5 mM magnesium chloride, 0.5 mM dithiothreitol) containing 0.1% Triton X-100 and a cocktail of protease inhibitors (17). Following extensive washes with phosphate-buffered saline, extracted cells were fixed with methanol and processed for indirect immunofluorescence.

In situ preparation of nuclear matrices. An established extraction procedure (33) was used to prepare RNA-containing nuclear matrices from cells grown on glass coverslips. Briefly, cells were treated with cytoskeleton buffer [10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 6.8), 100 mM sodium chloride, 300 mM sucrose, 3 mM magnesium chloride, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 4 mM vanadyl riboside complex (GIBCO-BRL), 0.5% Triton X-100, and protease inhibitors] for 3 min on ice and then with 100 μg of RNase-free DNase I (Boehringer Mannheim) per ml for 30 min at room temperature. Subsequently, cells were extracted first with 0.25 M ammonium sulfate and then with 2 M sodium chloride. The lack of DAPI staining in treated cells confirmed the efficient removal of nuclear DNA by this method. The nuclear matrix that remained following these extensive extractions was visualized by indirect immunofluorescence using the anti-NuMA monoclonal antibody Ab-1.

Western blot (immunoblot) analysis. WCL2, Cos-1, or stably transfected CHO cells grown on tissue culture plates were harvested, and nuclear matrices were prepared essentially as described above for cells grown on coverslips (33). Proteins, extracted from the resultant nuclear matrix pellet by using sodium dodecyl sulfate (SDS) sample buffer, were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting, using previously described conditions to visualize GR or SV40 TAg (86). Blots were also costained with either NuMA antibody Ab-1 or a commercially available lamin B antibody (Oncogene Science) to provide an internal control for recovery of nuclear matrix protein in separate samples.

RESULTS

Depletion of ATP from WCL2 cells. Given the apparent discrepancy in the energy requirements for steroid receptor nuclear export (32, 38, 49), we have used an indirect immunofluorescence assay to directly visualize the subcellular localization of two steroid receptors (PR and GR) in the same cell type (CHO) upon ATP depletion. In previous studies, sodium azide treatment had been shown to be effective for depletion of ATP from WCL2 cells (38), a CHO cell derivative that expresses stably transfected mouse GR (35). As shown in Fig. 1, ATP levels were depleted by greater than 95% upon treatment of WCL2 cells for 90 min with 10 mM sodium azide in the presence of 6 mM 2-deoxyglucose. Importantly, this method of ATP depletion is reversible, as ATP was restored to control levels when cells treated with sodium azide and 2-deoxyglucose for 90 min were returned to normal growth medium for 90 min (not shown). As an extended treatment with sodium azide (i.e., for >3 h) led to the accumulation of many cells with abnormal morphology and extensive cell death (not shown), we limited

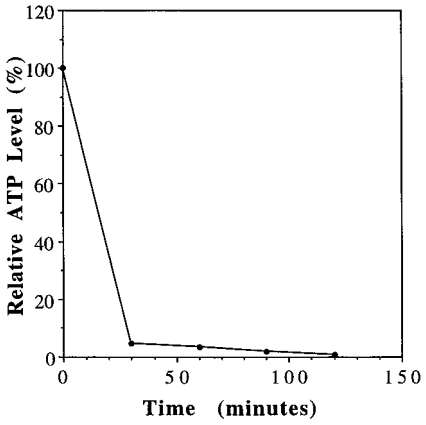

FIG. 1. ATP depletion in WCL2 cells. WCL2 cells were incubated at 37°C in ATP depletion medium as described in Materials and Methods. After 30, 60, and 90 min, ATP levels were measured from whole cell lysates by using an ATP Bioluminescence CLS kit and presented as percentage of ATP levels measured at time zero.

our analysis of steroid receptor nuclear export to energy deprivation conditions (i.e., 90-min treatments) that were readily reversible and not noticeably detrimental to cell viability.

GRs and PRs are retained within nuclei of ATP-depleted cells. Mouse GRs in WCL2 cells are predominantly localized within the nucleus when either ligand bound or unliganded (Fig. 2A and reference 64). As with all steroid receptors (11, 15, 32, 45), this localization does not reflect a static confinement of receptors to the nuclear compartment. GRs within WCL2 cells shuttle between the nuclear and cytoplasmic compartment (unpublished observations) but predominantly localize within the nucleus presumably because of their limited rate of nuclear export (16, 45). Orti et al. have postulated that GR nuclear export is an energy-requiring process, as judged from the biochemical detection of nuclear GRs in ATP-depleted cells (57), including WCL2 cells (38). GRs associated with the nucleus of ATP-depleted cells have been designated null GRs, given their deficiency in hormone binding (49). Using an ATP depletion method essentially identical to that used by Hu et al. (reference 38 and Fig. 1), we directly visualized nuclear null GRs by indirect immunofluorescence in ATP-depleted WCL2 cells (Fig. 2B). The nuclear retention of GRs upon ATP depletion is not a peculiarity of WCL2 cells or mouse GRs, as endogenous receptors in Dex-treated GrH2 rat hepatoma cells were also localized within nuclei upon ATP depletion (Fig. 2D).

In contrast to the hypothesis put forth by Orti et al. (57), Guiochon-Mantel et al. have postulated that nuclear export of PR, and perhaps other steroid receptors, is energy independent, given the observed redistribution of PRs from the nucleus to the cytoplasm under some conditions of energy deprivation (32). Using conditions that deplete >95% of cellular ATP (Fig. 1), we found that transiently transfected chicken PR, like mouse and rat GRs, remained associated with the nucleus of CHO cells (Fig. 2F). Since an extended period of ATP depletion had detrimental effects on CHO cell viability (see above), we did not feel it appropriate to examine GR and PR nuclear retention properties upon prolonged ATP depletion, as generally performed by others (32). Although we are therefore limiting our analysis to periods of ATP depletion that may be insufficient to reveal relatively slow nucleus-to-cytoplasm transport, we have been able to uncover a subnuclear trafficking pathway of GR that has a distinct ATP-dependent step (see below).

GRs within nuclei of ATP-depleted cells are resistant to extraction. The association of some nuclear proteins with distinct nuclear compartments is often reflected in their differential extraction properties. For example, an in situ hypotonic buffer-detergent extraction has been used to demonstrate the differential nuclear affinity of distinct phosphorylated isoforms of the retinoblastoma (Rb) protein (53). An identical in situ hypotonic buffer-detergent extraction of metabolically active WCL2 (Fig. 3C) and GrH2 (Fig. 3G) cells efficiently extracted ligand-bound nuclear GRs. Unliganded GRs which localize to the nucleus of WCL2 cells (64) were identically extracted under these conditions (not shown). In striking contrast, nuclear GRs in ATP-depleted WCL2 (Fig. 3D) and GrH2 (Fig. 3H) cells were generally resistant to this hypotonic buffer-detergent extraction. When ATP levels were restored to sodium azide-treated WCL2 cells, nuclear GRs regained their sensitivity to hypotonic buffer-detergent extraction (Fig. 4), demonstrating that nuclear receptors were not permanently trapped in an unextractable compartment. Importantly, cycloheximide was included in the growth medium to inhibit de novo protein synthesis during the restoration of cellular ATP (Fig. 4D). Thus, de novo protein synthesis was not required to restore the extractability of GRs, which previously resided within an unextractable nuclear compartment of ATP-depleted cells. This result is consistent with the observed recovery of GR hormone-binding activity in ATP-depleted cells in which ATP levels were likewise restored in the presence of cycloheximide (49).

GRs in ATP-depleted cells are bound to the nuclear matrix. The phosphorylated isoforms of nuclear Rb protein that resist extraction with hypotonic-detergent buffer are bound to the nuclear matrix (46). GRs have also been found to be associated with the nuclear matrix, although in contrast to other steroid receptors, their interaction with the matrix appears to be relatively weak and typically requires chemical cross-linking to be detected (42, 74). To assess whether unextractable GRs in ATP-depleted WCL2 cells are bound to the nuclear matrix, cells grown on coverslips were subjected to DNase I digestion and high-salt and detergent extraction in order to generate an RNA-containing nuclear matrix (33). WCL2 cells treated in this manner were devoid of DNA, as revealed by the lack of DAPI staining (not shown), but retained their nuclear matrix, as judged by the presence of immunoreactive NuMA (Fig. 5A and 5B). In indirect immunofluorescence micrographs, immunoreactive GRs were not detected in nuclear matrix preparations of metabolically active WCL2 cells (Fig. 5C), while GRs were readily detectable in the nuclear matrix preparations from ATP-depleted cells (Fig. 5D). Confocal microscopic analysis revealed that GR was associated with the nuclear matrix throughout all planes of the nucleus (Fig. 6) and not just the peripheral, lamin-enriched region of the nuclear matrix (33). Thus, GRs appear to be confined to the nucleus of ATP-depleted cells as a result of their increased association with the nuclear matrix. As shown below (Fig. 9 and 10), sensitive Western blot analyses enable quantitative comparisons to be made between GR bound to the nuclear matrix of metabolically active and ATP-depleted cells.

We consider it unlikely that our ATP depletion paradigm leads to an irreversible collapse of nuclear proteins onto the nuclear matrix for the following reasons. First, GRs which were bound to the matrix of ATP-depleted cells regain the ability to be extracted by hypotonic buffer when ATP levels are restored (Fig. 4). Second, a rat GR NLS–β-Gal chimera that constitutively localizes within nuclei (58) was sensitive to hypotonic buffer extraction in both metabolically active and ATP-depleted cells (Fig. 7). This result also establishes that the 27-amino-acid constitutive NLS of the rat GR (58) is not sufficient

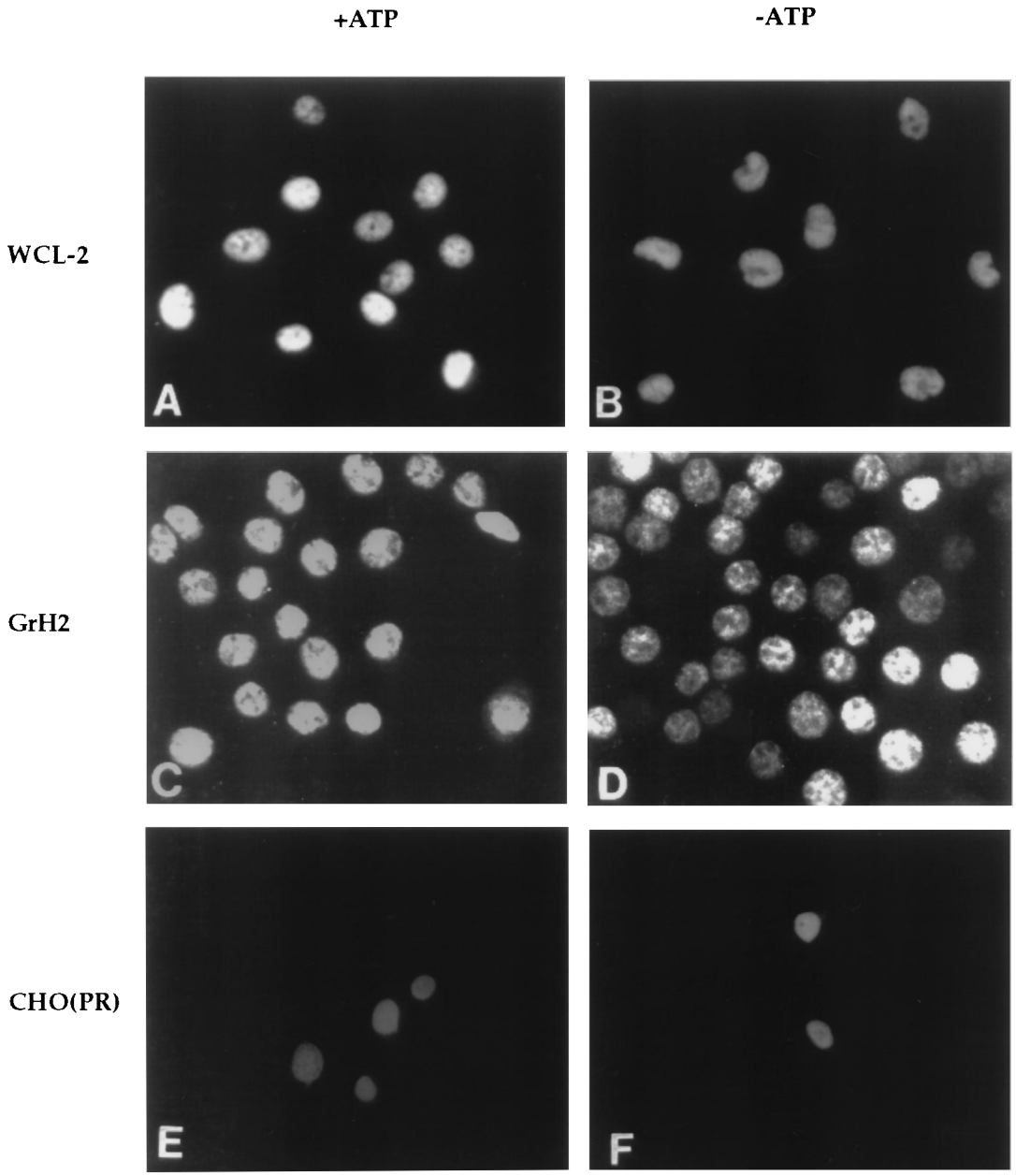

FIG. 2. GRs and PRs are retained within nuclei of ATP-depleted cells. WCL2 (A and B), GrH2 (C and D), and transiently transfected CHO (E and F) cells grown on coverslips were either untreated (A, C, and E) or treated with 10 mM sodium azide for 90 min (B, D, and F), fixed with methanol, and subjected to indirect immunofluorescence, using either monoclonal antibody BuGR2 to detect GR (A to D) or monoclonal antibody PR22 to detect transiently transfected chicken PR (E and F). Dex (1 μM) was added to WCL2 and GrH2 cells, although GRs in ATP-depleted cells are known to lack hormone-binding activity.

for targeting the receptor to the nuclear matrix, in agreement with results obtained with chemically cross-linked nuclear matrix preparations (74).

Nuclear matrix binding of SV40 TAg is unaltered by ATP depletion. As an additional assessment for effects of ATP depletion on the composition or functioning of the nuclear matrix, we examined whether the association of another nuclear protein with the nuclear matrix was altered upon ATP depletion. SV40 TAg has been shown to distribute between various subnuclear compartments (11, 69). In particular, biochemical fractionations revealed a distinct subpopulation of SV40 TAg that associates with the nuclear matrix (14). As shown in Fig. 8F, a significant fraction of nuclear SV40 TAg in Cos-1 cells, as visualized by indirect immunofluorescence, resists hypotonic buffer-detergent extraction and is likely nuclear matrix bound. Interestingly, the relative proportion of nuclear SV40 TAg that is associated with the nuclear matrix does not appear to be dramatically altered upon ATP depletion of Cos-1 cells (Fig. 8H). Western blot analysis corroborated that the fraction of nuclear SV40 TAg bound to the nuclear matrix was not quantitatively altered upon ATP depletion (Fig. 9A). Thus, it appears that the increased association of nuclear proteins with the matrix is not a universal consequence of ATP depletion and may apply to a select subset of nuclear proteins, such as the GR and possibly other steroid receptors.

In confirmation of previous biochemical results of Munck

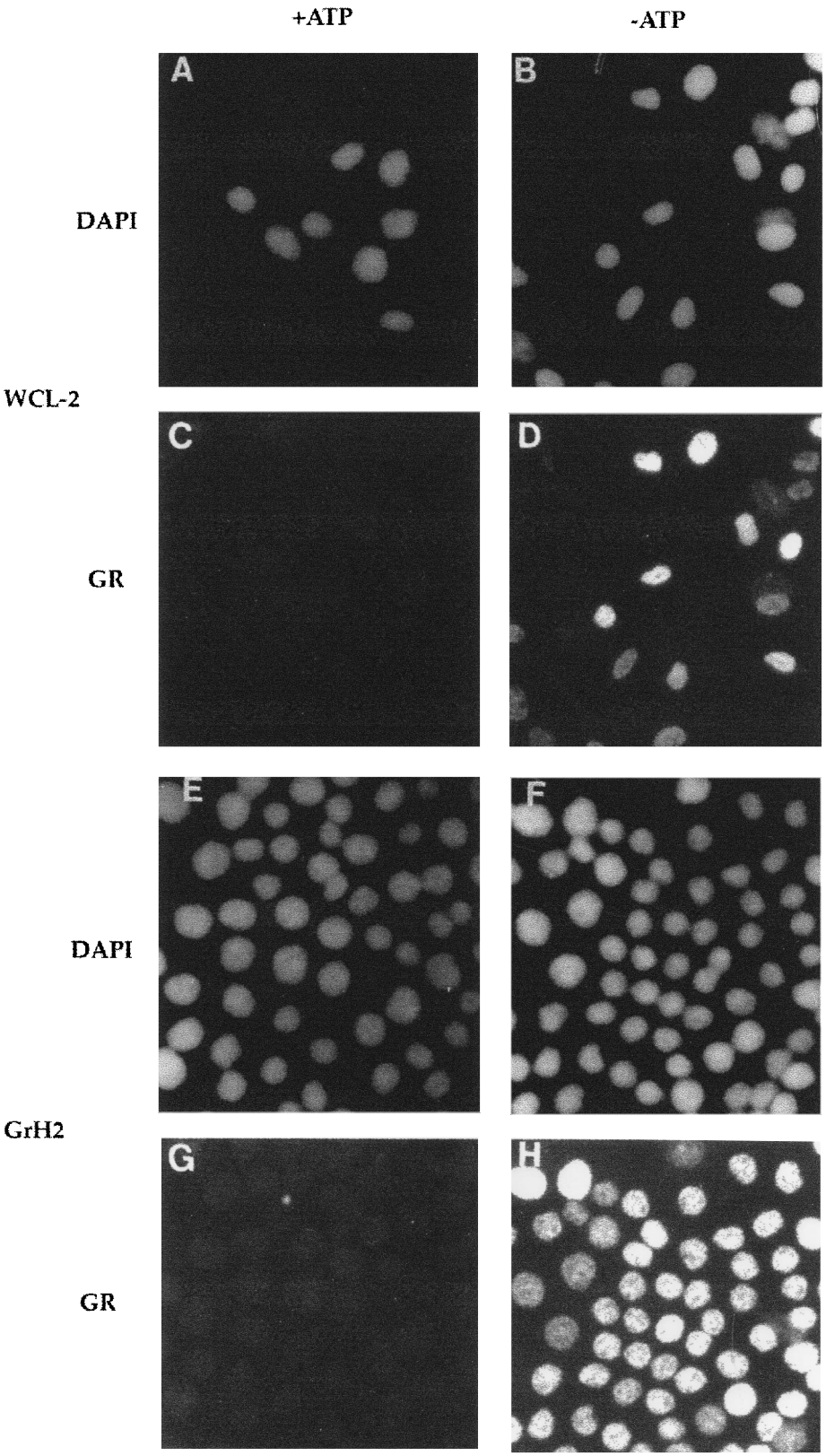

FIG. 3. GRs within nuclei of ATP-depleted WCL2 and GrH2 cells are resistant to extraction. WCL2 (A to D) and GrH2 (E to H) cells grown on coverslips, either untreated (A, C, E, and G) or treated with 10 mM sodium azide for 90 min (B, D, F, and H), were extracted in situ with hypotonic buffer containing 0.1% Triton X-100 as described in Materials and Methods. Nuclei were visualized by DAPI staining (A, B, E, and F), and GRs were detected by indirect immunofluorescence, using anti-GR monoclonal antibody BuGR2 (C, D, G, and H). Dex (1 μM) was added to all cultures.

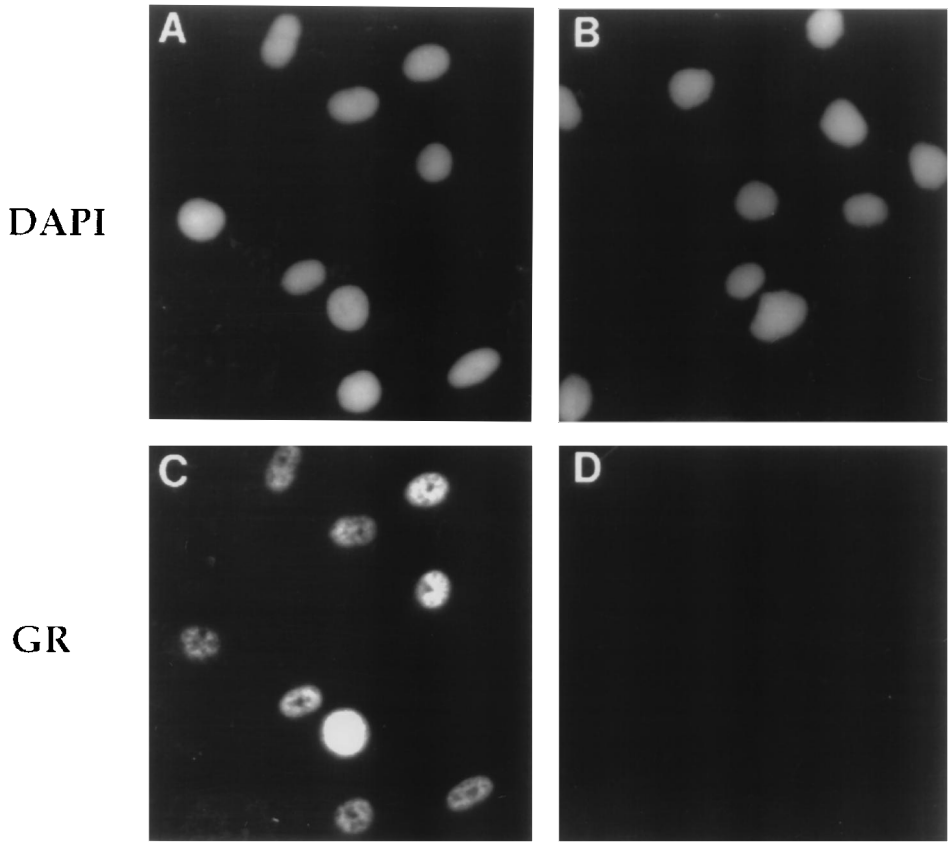

FIG. 4. Recovery of GR nuclear extractability does not require de novo protein synthesis. WCL2 cells were treated with ATP depletion medium containing 10 mM sodium azide for 90 min and then returned to normal medium plus 100 μg of cycloheximide per ml and 1 μM Dex. Cells were either fixed directly (A and C) or extracted with hypotonic buffer and then extracted (B and D). Nuclei were visualized by DAPI staining (A and B), and GRs were detected by indirect immunofluorescence, using anti-GR monoclonal antibody BuGR2 (C and D).

and coworkers (38, 49) and our own immunofluorescence studies (Fig. 5), Western blot analysis likewise revealed the increased association of GRs with the nuclear matrix upon ATP depletion (Fig. 9B). The sensitivity of the Western blot permitted the detection of a minor population of GR that was bound to the nuclear matrix of metabolically active WCL2 cells (Fig. 9B) which was not easily detectable in indirect immunofluorescence micrographs (e.g., Fig. 5C). In addition to differences in sensitivity between the enhanced chemiluminescence method used in Western blotting and indirect immunofluorescence, the detection of nuclear matrix-bound GRs has been shown to be highly dependent on extraction methods (74). For example, nuclear matrix binding of human GR in transfected Cos-1 cells required the use of sodium tetrathionate during extraction to cross-link receptors to the matrix (74). Irrespective of extraction methods used, a number of independent studies have clearly established the association of some fraction of GR with the nuclear matrix in both cultured cells (74) and intact tissue (42, 75).

The rat GR DBD is required for nuclear matrix binding in metabolically active and ATP-depleted cells. Despite the apparent selectivity of ATP depletion effects (see above), it remains formally possible that the increased association of GRs with the nuclear matrix involves novel interactions which become apparent only upon energy deprivation. Such novel encounters with the matrix might be revealed by the comparison of domain requirements for GR nuclear matrix binding in metabolically active and ATP-depleted cells. The human GR DBD, and to a lesser extent its LBD, have been shown to be required for nuclear matrix binding under chemical cross-linking conditions (74). We therefore generated a number of stably transfected CHO cell lines expressing GR deletion mutants and compared their nuclear matrix-binding capacities in metabolically active and ATP-depleted cells, using Western blot analysis. All blots were costained with a lamin B antibody to correct for any variations in nuclear matrix recovery between different samples. In addition, the amount of GR present in equivalent amounts of crude cell extract was assessed for each cell line.

As shown in Fig. 10, ATP depletion led to increased nuclear matrix binding of GR deletion mutants that lack either 239 amino acids from the carboxy terminus (compare lanes 5 and 6) or 407 amino acids from the amino terminus (compare lanes 8 and 9). The apparent decline in nuclear matrix binding, in metabolically active cells, of carboxy-terminally (i.e., VAN556) and amino-terminally (i.e., VA407C) deleted GRs (lanes 5 and 8, respectively) is difficult to confirm given the low levels of GR recovered in our matrix preparations. When human GR interactions with the nuclear matrix are stabilized by chemical cross-linking, deletion of the receptor amino-terminal domain does not appear to affect its binding to the matrix, while deletion of the carboxy-terminal LBD leads to a slight reduction in matrix binding (74). In this cross-linking paradigm, nuclear matrix binding of human GR was abolished upon mutation of the DBD (74). In agreement with these results, nuclear matrix binding, in metabolically active cells, of an LBD-deleted rat

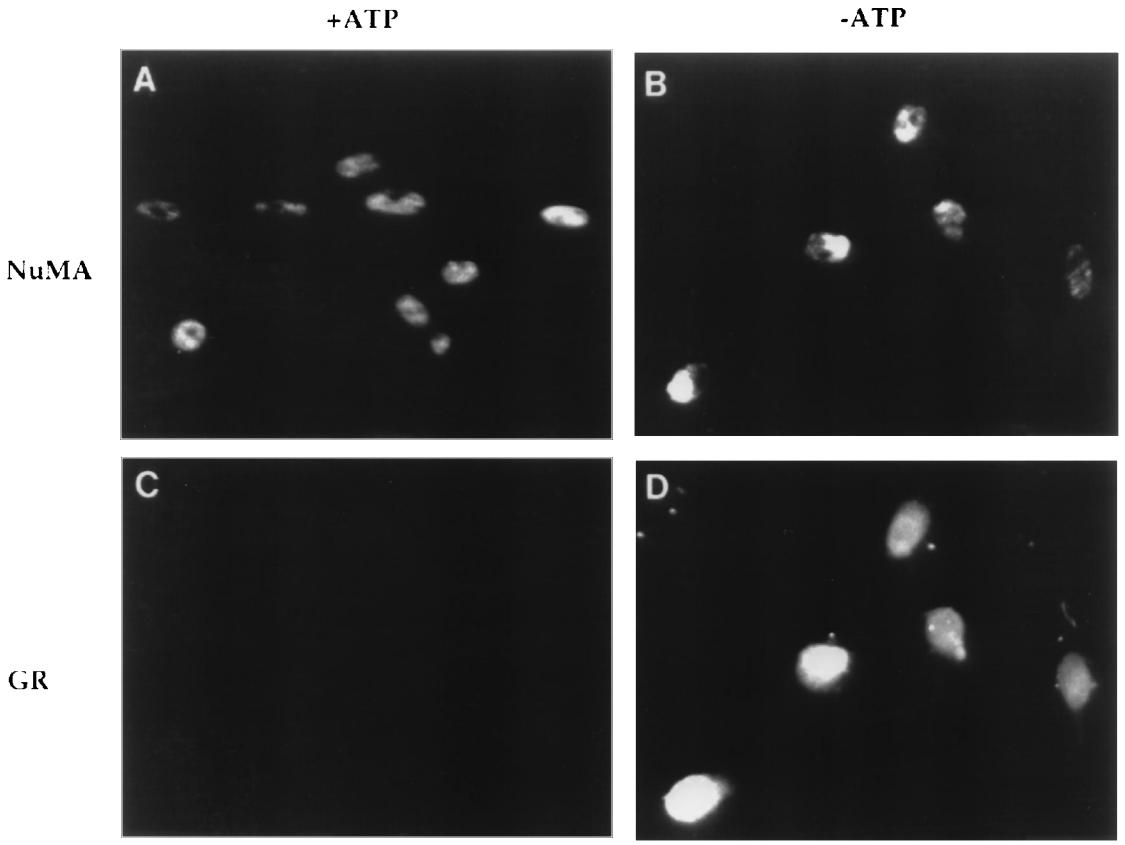

FIG. 5. GRs in ATP-depleted WCL2 cells are bound to the nuclear matrix. WCL2 cells grown on coverslips in the presence of 1 μM Dex were either untreated (A and C) or treated with 10 mM sodium azide for 90 min to deplete ATP (B and D). Cells were subsequently extracted with DNase I and high salt to generate an RNA-containing nuclear matrix, fixed with methanol, and subjected to indirect immunofluorescence to visualize GR (C and D) and the NuMA (A and B) in identical fields of cells.

GR was abolished upon the introduction of a 38-amino-acid internal deletion within its DBD (lane 11). Interestingly, the GR DBD deletion mutant was also not detected in nuclear matrix preparations from ATP-depleted cells (lane 12). Thus, an intact DBD is required for ATP depletion to elicit its stabilizing effect on GR-nuclear matrix interactions, which is manifested even in the absence of GR domains that may contribute to matrix binding (74). This result provides strong support for the notion that ATP depletion does not merely expose the receptor to interactions which are never encountered under normal physiological conditions but more likely alters the dynamics of such interactions.

DISCUSSION

A clear consensus concerning the nature of common and unique aspects of inward and outward trafficking through the nuclear pore complex (NPC) has yet to emerge, as conflicting results have been generated regarding such fundamental issues as the signal sequence (31, 51, 65, 79) and energy (32, 79) requirements of nuclear protein export. For example, prior to this report, it was not known whether a single class of closely related proteins, the steroid receptors, utilize the energy derived from ATP hydrolysis for nuclear export (32, 49). The results that we have obtained indicate that the debate over this issue has overlooked the important prerequisite that nuclear proteins destined for export must first gain access to the NPC. Thus, upon depletion of ATP in vivo, GRs and PRs remain bound to the nuclear matrix and, as a result, are not presented to the nuclear export machinery. Our results are therefore consistent with the hypothesis that nuclear retention can restrict nuclear export (65) but demonstrate that for steroid receptors, ATP relieves that restriction through facilitating the release of receptors from the nuclear matrix.

Our experiments do not address whether ATP is required for the translocation of steroid receptors through the NPC. Recent in vitro experiments demonstrate that nuclear export of an NLS conjugate requires GTP but not ATP (54), implying that the translocation through the NPC of natural substrates, such as steroid receptors, may not utilize the energy of ATP hydrolysis. The fact that NLS conjugates are most likely not bound to the nuclear matrix (reference 74 and this report) provides a likely explanation for their efficient in vivo (31) and in vitro (54) ATP-independent nuclear export. In the absence of some assessment of the functional or structural integrity of the nuclear matrix, studies of nuclear export involving prolonged ATP depletion (31) must be interpreted with caution. The nuclear matrix association of GR under our conditions of ATP depletion is completely reversible in vivo, which provides some assurance that the nuclear matrix in these cells is not permanently disabled. Likewise, the fact that nuclear matrix binding of SV40 TAg (14) is not obviously affected by ATP depletion demonstrates that wholesale collapse of nuclear proteins onto the matrix does not occur under these conditions.

The association of steroid receptors with the nuclear matrix has been the subject of considerable investigations over the

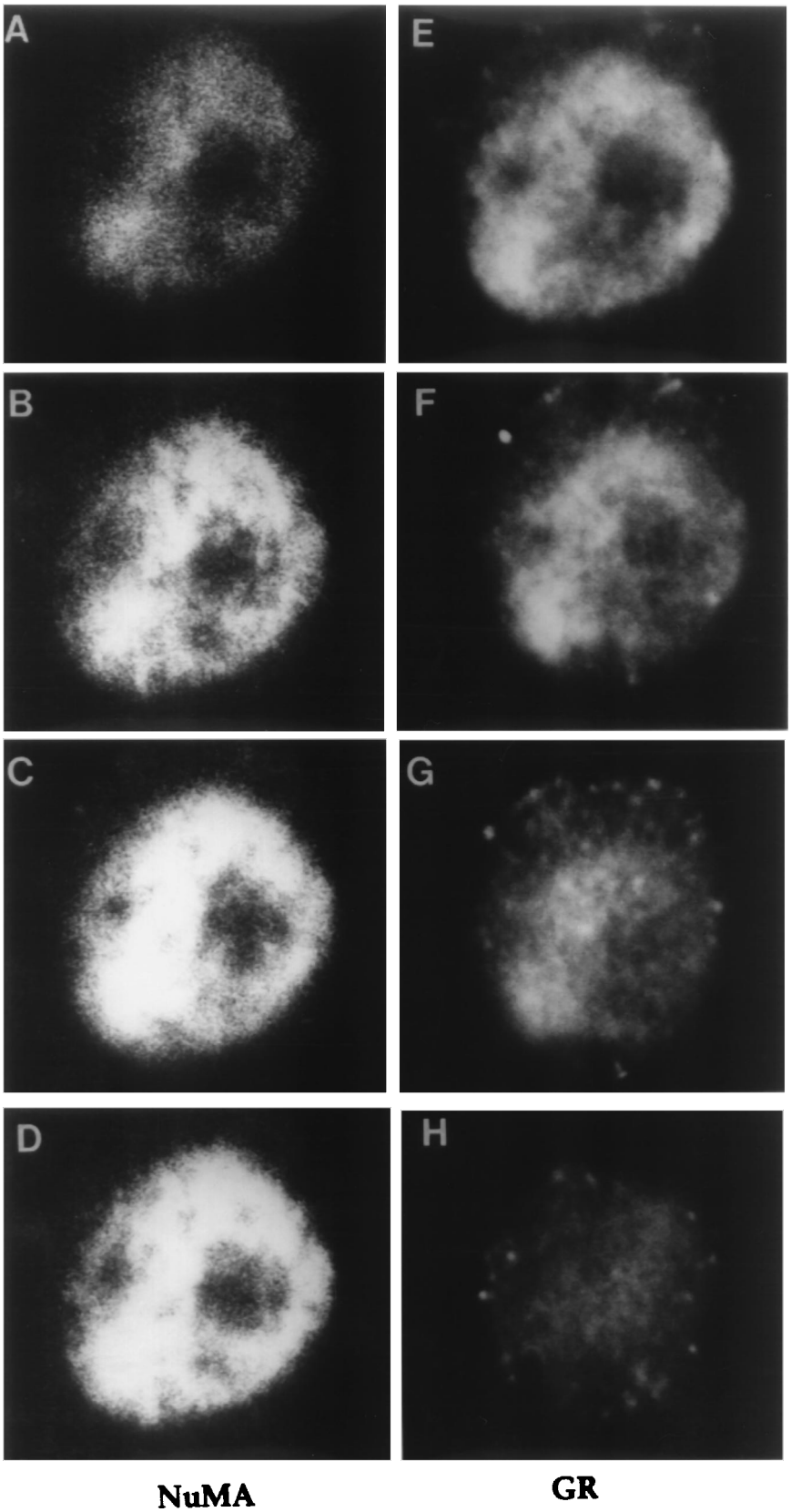

FIG. 6. Confocal microscopic images of nuclear matrix-associated GR and NuMA in ATP-depleted WCL2 cells. WCL2 cells treated with 10 mM sodium azide for 90 min to deplete ATP were extracted with DNase I and high salt to generate an RNA-containing nuclear matrix. Following methanol fixation and indirect immunofluorescence staining, NuMA (A to D) and GR (E to H) bound to the nuclear matrix were visualized in consecutive 0.7-μm optical sections (A to D and E to H), using a Zeiss laser scanning confocal microscope.

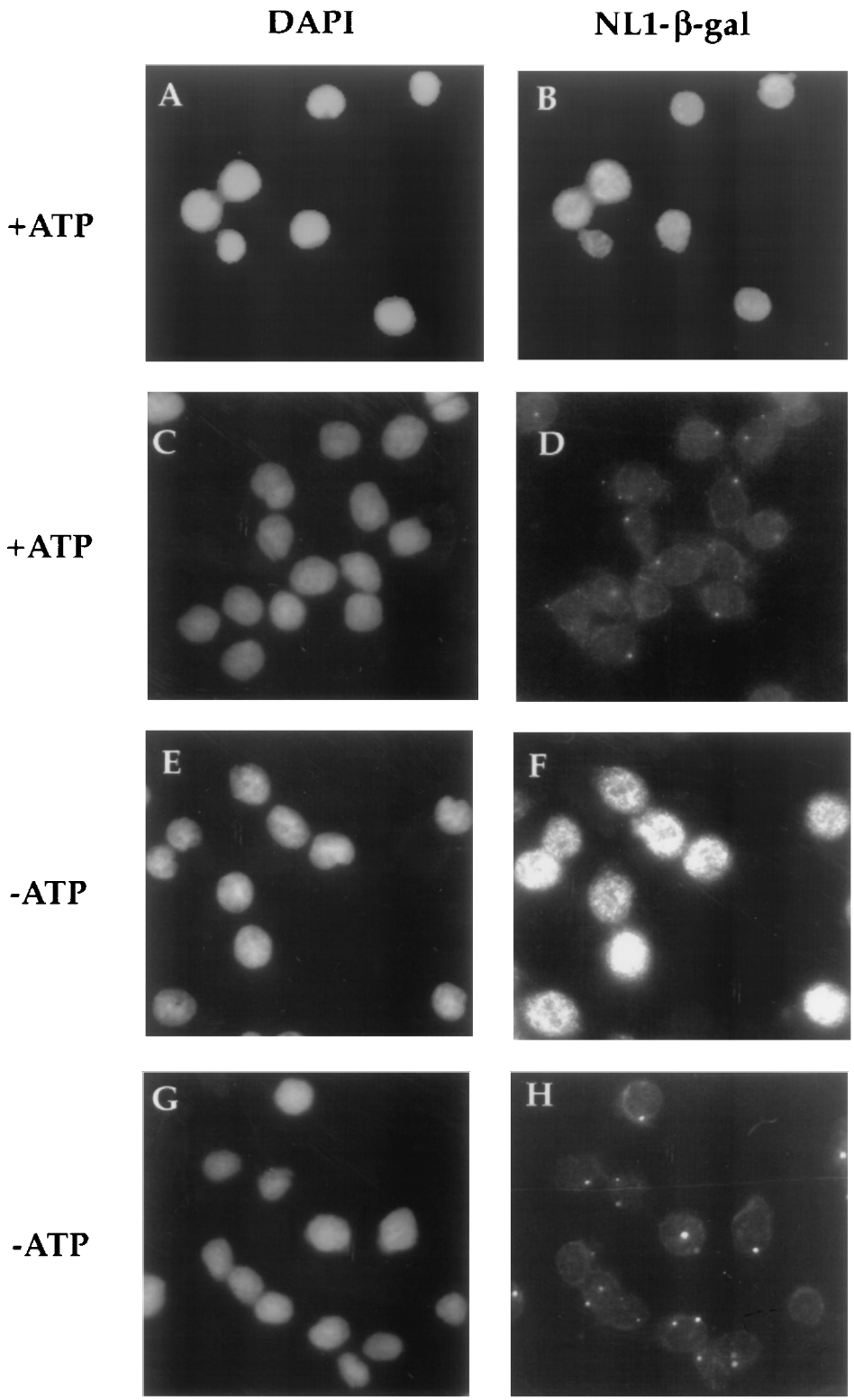

FIG. 7. ATP depletion does not alter the extractability of a nuclear GR NLS–β-Gal chimera. CHO cells stably expressing a GR NLS–β-Gal chimera were grown on coverslips and either untreated (A to D) or treated with 10 mM sodium azide for 90 min (E to H). Cells were then either directly fixed (A, B, E, and F) or extracted in situ with hypotonic buffer containing 0.1% Triton X-100 prior to fixation (C, D, G, and H). Nuclei were visualized by DAPI staining (A, C, E, and G), and NL1–β-Gal protein was detected by indirect immunofluorescence, using a commercially available anti-β-Gal monoclonal antibody (B, D, F, and H).

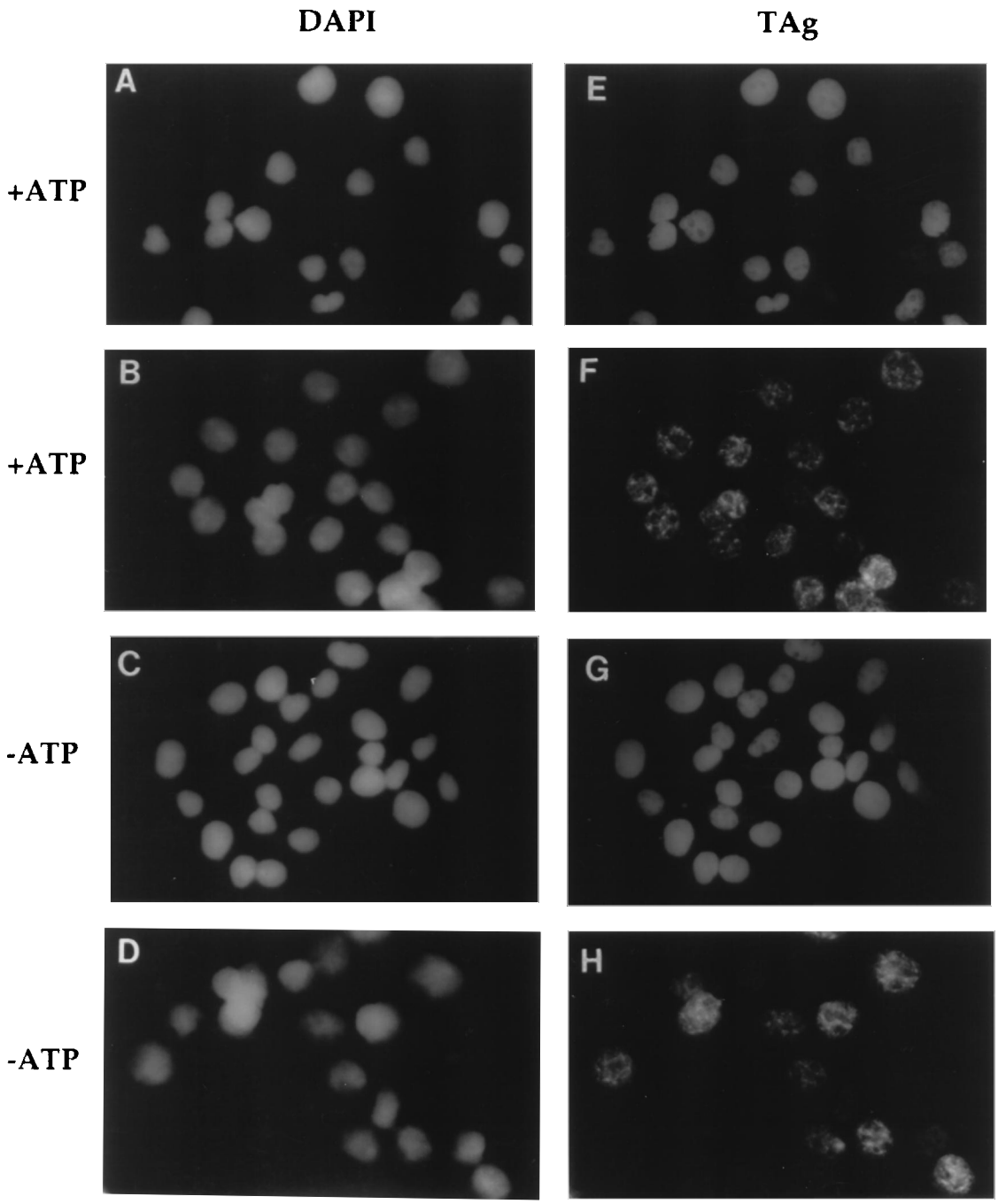

FIG. 8. The subnuclear distribution of SV40 TAg is unaltered by ATP depletion. Cos-1 cells grown on coverslips were either untreated (A, B, E, and F) or treated with 10 mM sodium azide for 90 min to deplete ATP (C, D, G, and H). Cells were either fixed directly (A, C, E, and G) or extracted with hypotonic buffer and Triton X-100 prior to fixation (B, D, F, and H). Nuclei were visualized by DAPI staining (A to D), and SV40 TAg was detected by indirect immunofluorescence, using anti-SV40 TAg hamster polyclonal antibody HAT-1 (E to H).

past 15 years (2, 3, 27). The in vitro binding of steroid receptors to the nuclear matrix is saturable, high affinity, and hormone dependent (2, 50, 66). Although nuclear matrix binding of human AR is mainly directed by its LBD (74), the DBD appears to possess the predominant nuclear matrix-binding domain of human (74) and rat (this study) GRs. The essential role of the GR DBD in nuclear matrix binding has been established both in the presence and in the absence of chemical cross-linkers, and in metabolically active and ATP-depleted cells, implying that these different cell growth or extraction conditions do not fundamentally alter the nature of receptor interactions with the matrix. Importantly, the results of our studies reveal the dynamic nature of steroid receptor-nuclear matrix interactions and demonstrate an ATP requirement for receptor release from, but not binding to, the nuclear matrix.

The nuclear matrix provides a framework for organizing large macromolecular assemblies that carry out the fundamen-

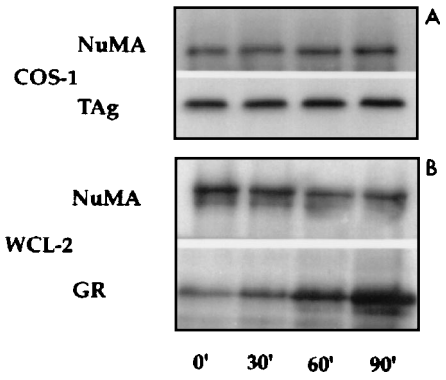

FIG. 9. Increase in GR, but not SV40 TAg, association with the nuclear matrix of ATP-depleted cells. WCL2 or Cos-1 cells were treated with 10 mM sodium azide for the lengths of time indicated (0, 30, 60, and 90 min), and nuclear matrices were prepared as described in Materials and Methods. Proteins obtained from the nuclear matrix pellet following solubilization with SDS sample buffer were separated by SDS-PAGE, using 7.5% polyacrylamide. Western blots were costained with anti-NuMA monoclonal antibody Ab-1 and either anti-GR monoclonal antibody BuGR2 (WCL2 cells; B) or an anti-SV40 TAg monoclonal antibody (Cos-1 cells; A).

tal nuclear processes of DNA replication, transcription, and splicing. Given the enormous variety of developmental, cell-type-specific, and hormonal factors that influence these processes, it seems likely that the nuclear matrix maintains some degree of plasticity in its structure and composition. The composition of the low-abundance NuMA protein varies between different cell types (23, 28). In addition, the association of specific transcription factors with the nuclear matrix has been shown to vary between different cell types (76). As some transcription factors partition between the nuclear matrix and soluble compartments of the nucleus (1, 73), it seems likely that nuclear matrix binding of transcription factors is not a static process but includes the dynamic exchange between distinct nuclear compartments. For steroid receptor proteins whose interaction with the nuclear matrix is conditional (i.e., hormone dependent), the efficiency of their binding to and release from the matrix may exert a considerable influence on their ability to scan the genome and locate appropriate target sites.

Have any mechanistic insights been uncovered concerning the conditional association of transcription factors with the nuclear matrix? The tumor suppressor Rb protein is associated with the nuclear matrix predominantly during the G_1 phase of the cell cycle, a time at which Rb expresses its growth-suppressing activities (46). Rb which is associated with the nuclear matrix is relatively hypophosphorylated, but as cells progress through G_1, the extent of Rb phosphorylation increases coincident with its release from the nuclear matrix (52, 53). In vitro, the release of nuclear matrix-bound Rb is triggered upon its phosphorylation by cyclin-dependent protein kinases (52), suggesting that differential phosphorylation of Rb plays a direct role in regulating its partitioning between different nuclear compartments. Rb binds both to the abundant nuclear matrix lamin A and C proteins (46) and to p84, a low-abundance nuclear matrix protein that colocalizes to sites of RNA processing (19). While the association of hypophosphorylated Rb with p84 appears specifically during G_1, suggesting that this interaction may be relevant to Rb function, additional factors appear to contribute to the nuclear matrix binding of Rb (19, 46).

We consider it unlikely that the increased association of GR with the nuclear matrix is due to alterations in its phosphorylation. Although GR in ATP-depleted WCL2 cells has been found to be dephosphorylated at two serine residues (positions 220 and 234), mutations of those serines to alanine residues did not alter the relative fraction of nuclear receptors that resists high-salt and detergent extraction (38). We have corroborated these results through the analysis of a GR amino-terminal deletion mutant, which includes these and most other receptor phosphorylation sites (7), and a receptor derivative with point mutations that eliminate GR phosphorylation at seven sites (unpublished observations). Analogous to results obtained with wild-type GR, relatively low levels of phosphorylation-defective GRs are bound to the matrix of metabolically active cells, while a dramatic increase in matrix binding occurs upon ATP depletion.

It seems likely that not all nuclear matrix associations are as dynamic as those involving GR. The association of TAg with the matrix does not appear to change as dramatically as that of GR. However, it is possible that under conditions of viral DNA replication, TAg shifts compartments as the availability, structure, composition, or compartmentalization of its target sites changes. During adenovirus infection, the spliceosome assembly factor SC35 apparently redistributes from discrete speckle domains (68) within the nucleus to sites of adenovirus transcription (41). Although the validity of this observation has recently been questioned (88), the nuclear distribution of SC35 has also been shown to change in response to overall inhibition of RNA polymerase II-directed transcription (9) or passage through distinct phases of the cell cycle (30). Interestingly, the cell cycle-specific alteration in SC35 nuclear compartmentalization may be mediated by a specific cell cycle-regulated serine kinase which directly phosphorylates SC35 in vitro (30). Clearly, there must be mechanisms that operate to facilitate the efficient trafficking of macromolecules throughout the nu-

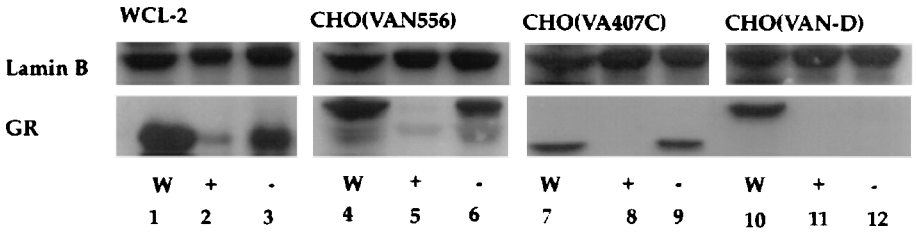

FIG. 10. The rat GR DBD is required for nuclear matrix binding in metabolically active and ATP-depleted cells. WCL2 or stably transfected CHO cells expressing various GR mutants were either maintained under normal growth conditions (lanes 1, 2, 4, 5, 7, 8, 10, and 11) or treated with 10 mM sodium azide for 90 min (lanes 3, 6, 9, and 12) to deplete cellular ATP. GR derivatives tested include wild-type receptor (WCL-2; lanes 1 to 3), a carboxy-terminal deletion (VAN556; lanes 4 to 6), an amino-terminal deletion (VA407C; lanes 7 to 9), and a combined carboxy-terminal, internal DBD deletion (VAN-D; lanes 10 to 12). Proteins obtained from either whole cells (lanes 1, 4, 7, and 10) or a nuclear matrix preparation (lanes 2, 3, 5, 6, 8, 9, 11, and 12) were solubilized with SDS sample buffer and separated by SDS-PAGE, using 10% polyacrylamide. Western blots were costained with an anti-lamin B monoclonal antibody (top) and anti-GR monoclonal antibody BuGR2 (bottom).

cleus as different demands are placed on the transcriptional, splicing, and replication machineries.

Steroid receptors are often constrained to function as hormone-dependent, and often cell-type-specific, regulators of transcription (83). How do the receptors rapidly locate their specific target sites when faced with an overwhelming excess of potential low-affinity targets? This question has been the subject of considerable debate ever since the realization that nuclear hormone receptors directly mediate many of the genomic actions of steroid hormones (84, 85). We now put forth the hypothesis that the rapid binding and release of steroid receptors from the nuclear matrix allow them to effectively scan the genome in search of specific target sites. The hunt by receptors for cell-type-specific genes may be facilitated by the specific association of either cell-type-specific transcription factors (20) or the genes themselves (25, 27) with specific regions of the matrix. As suggested previously (2), receptors may additionally be targeted to these active genomic sites by specific acceptor proteins. Our model is an elaboration of the previously proposed nuclear matrix acceptor hypothesis but adds the notion that the search for such sites is a dynamic one that utilizes the energy of ATP hydrolysis. It is conceivable that specific chaperone proteins assist in this novel subnuclear trafficking pathway and, analogous to their cytoplasmic counterparts (26, 34), use ATP binding and/or hydrolysis to regulate their ability to deliver proteins to and from distinct subnuclear compartments.

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REFERENCES

1. **Bagchi, M., A. van Wijnen, M. Katar, H. Merriman, J. Lian, J. Stein, G. Stein, and H. Maisel.** 1995. Sequence-specific DNA binding activities of nuclear matrix proteins of mammalian lens epithelial cells. J. Cell. Biochem. **58:**1–5.
2. **Barrack, E. R.** 1987. Localization of steroid hormone receptors in the nuclear matrix, p. 86–127. *In* C. R. Clark (ed.), Steroid hormone receptors. Their intracellular localisation. Ellis Horwood Ltd., Chichester, England.
3. **Barrack, E. R., and D. S. Coffey.** 1980. The specific binding of estrogens and androgens to the nuclear matrix of sex responsive tissues. J. Biol. Chem. **255:** 7265–7275.
4. **Bataille, N., T. Helser, and H. M. Fried.** 1990. Cytoplasmic transport of ribosomal subunits microinjected into the *Xenopus laevis* oocyte nucleus: a generalized facilitated process. J. Cell Biol. **111:**1571–1582.
5. **Berezney, R.** 1991. The nuclear matrix: a heuristic model for investigating genomic organization and function in the cell nucleus. J. Cell. Biochem. **47:** 109–123.
6. **Bidwell, J. P., A. J. van Wijnen, A. G. Fey, S. Dworetzky, S. Penman, J. L. Stein, J. B. Lian, and G. S. Stein.** 1993. Osteocalcin gene promoter-binding factors are tissue-specific nuclear matrix components. Proc. Natl. Acad. Sci. USA **90:**3162–3166.
7. **Bodwell, J. E., E. Orti, J. M. Coull, D. J. C. Pappin, D. B. Mendel, L. I. Smith, and F. Swift.** 1991. Identification of phosphorylated sites in the mouse glucocorticoid receptor. J. Biol. Chem. **266:**7549–7555.
8. **Borer, R. A., C. F. Lehner, H. M. Eppenberger, and E. A. Nigg.** 1989. Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell **56:**379–390.
9. **Bregman, D. B., L. Du, S. van der Zee, and S. L. Warren.** 1995. Transcription-dependent redistribution of the large subunit of RNA polymerase II to discrete nuclear domains. J. Cell Biol. **129:**287–298.
10. **Cadepond, F., J. M. Gasc, F. Delahaye, N. Jibard, G. G. Schweizer, M. I. Segard, R. Evans, and E. E. Baulieu.** 1992. Hormonal regulation of the nuclear localization signals of the human glucocorticosteroid receptor. Exp. Cell Res. **201:**99–108.
11. **Chandran, U. R., and D. B. DeFranco.** 1992. Internuclear migration of chicken progesterone receptor, but not simian virus-40 large tumor antigen, in transient heterokaryons. Mol. Endocrinol. **6:**837–844.
12. **Cidlowski, J. A., D. L. Bellingham, F. E. Powell-Oliver, D. B. Lubahn, and M. Sar.** 1990. Novel antipeptide antibodies to the human glucocorticoid receptor: recognition of multiple distinct receptor forms *in vitro* and distinct localization of cytoplasmic and nuclear receptors. Mol. Endocrinol. **4:**1427–1437.
13. **Ciejek, E., M. Tsai, and B. W. O'Malley.** 1983. Actively transcribed genes are associated with the nuclear matrix. Nature (London) **306:**607–609.
14. **Covey, L., Y. Choi, and C. Prives.** 1984. Association of simian virus 40 T antigen with the nuclear matrix of infected and transformed monkey cells. Mol. Cell. Biol. **4:**1384–1392.
15. **Dauvois, S., R. White, and M. G. Parker.** 1993. The antiestrogen ICI 182780 disrupts estrogen receptor nucleocytoplasmic shuttling. J. Cell Sci. **106:**1377–1388.
16. **DeFranco, D. B., A. P. Madan, Y. Tang, U. R. Chandran, N. Xiao, and J. Yang.** 1995. Nucleocytoplasmic shuttling of steroid receptors. Vitam. Horm. **51:**315–338.
17. **DeFranco, D. B., M. Qi, K. C. Borror, M. J. Garabedian, and D. L. Brautigan.** 1991. Protein phosphatase types 1 and/or 2A regulate nucleocytoplasmic shuttling of glucocorticoid receptors. Mol. Endocrinol. **5:**1215–1228.
18. **Dickinson, L. A., T. Joh, Y. Kohwi, and T. Kohwi-Shigematsu.** 1992. A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition. Cell **70:**631–645.
19. **Durfee, T., M. A. Mancini, D. Jones, S. J. Elledge, and W.-H. Lee.** 1994. The amino-terminal region of the retinoblastoma gene product binds to a novel nuclear matrix protein that co-localizes to centers for RNA processing. J. Cell Biol. **127:**609–622.
20. **Dworetzky, S. I., K. L. Wright, E. G. Fey, S. Penman, J. B. Lian, J. L. Stein, and G. S. Stein.** 1992. Sequence-specific DNA binding proteins are components of a nuclear matrix attachment site. Proc. Natl. Acad. Sci. USA **89:** 4178–4182.
21. **Fey, E. G., P. Bangs, C. Sparks, and P. Odgren.** 1991. The nuclear matrix: defining structural and functional roles. Crit. Rev. Eukaryotic Gene Expr. **1:** 127–144.
22. **Fey, E. G., G. Krochmalnic, and S. Penman.** 1986. The non-chromatin substructures of the nucleus: the ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. J. Cell Biol. **102:**1654–1665.
23. **Fey, E. G., and S. Penman.** 1989. Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc. Natl. Acad. Sci. USA **85:**121–125.
24. **Gametchu, B., and R. W. Harrison.** 1984. Characterization of a monoclonal antibody to the rat liver glucocorticoid receptor. Endocrinology **114:**274–288.
25. **Gasser, S. M., and U. K. Laemmli.** 1986. Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated *Drosophila melanogaster* genes. Cell **46:**521–530.
26. **Georgopoulos, C., and W. J. Welch.** 1993. Role of the major heat shock proteins as molecular chaperones. Annu. Rev. Cell Biol. **9:**601–634.
27. **Getzenberg, R. H.** 1994. Nuclear matrix and the regulation of gene expression: tissue specificity. J. Cell. Biochem. **55:**22–31.
28. **Getzenberg, R. H., and D. S. Coffey.** 1990. Tissue specificity of the hormonal response in sex accessory tissues is associated with nuclear matrix protein patterns. Mol. Endocrinol. **4:**1336–1342.
29. **Godowski, P. J., S. Rusconi, R. Miesfeld, and K. R. Yamamoto.** 1987. Glucocorticoid receptor mutants that are constitutive activators of transcriptional enhancement. Nature (London) **325:**365–368.
30. **Gui, J.-F., W. S. Lane, and X.-D. Fu.** 1994. A serine kinase regulates intracellular localization of splicing factors in the cell cycle. Nature (London) **369:** 678–682.
31. **Guiochon-Mantel, A., K. Delabre, P. Lescop, and E. Milgrom.** 1994. Nuclear localization signals also mediate the outward movement of proteins from the nucleus. Proc. Natl. Acad. Sci. USA **91:**7179–7183.
32. **Guiochon-Mantel, A., P. Lescop, S. Christin-Maitre, H. Loosefelt, M. Perrot-Applanat, and E. Milgrom.** 1991. Nucleocytoplasmic shuttling of the progesterone receptor. EMBO J. **10:**3851–3859.
33. **He, D., J. A. Nickerson, and S. Penman.** 1990. Core filaments of the nuclear matrix. J. Cell Biol. **110:**569–580.
34. **Hendrick, J. P., and F.-U. Hartl.** 1993. Molecular chaperone functions of heat-shock proteins. Annu. Rev. Biochem. **62:**349–384.
35. **Hirst, M. A., J. P. Northrop, M. Danielsen, and G. M. Ringold.** 1990. High level expression of wild type and variant mouse glucocorticoid receptors in chinese hamster ovary cells. Mol. Endocrinol. **4:**162–170.
36. **Howard, K. J., S. J. Holley, K. R. Yamamoto, and C. W. Distelhorst.** 1990. Mapping the HSP90 binding region of the glucocorticoid receptor. J. Biol. Chem. **265:**11928–11935.
37. **Hozak, P., A. B. Hassan, D. A. Jackson, and P. R. Cook.** 1993. Visualization of replication factories attached to the nucleoskeleton. Cell **73:**361–373.
38. **Hu, L.-M., J. Bodwell, J.-M. Hu, E. Orti, and A. Munck.** 1994. Glucocorticoid receptors in ATP-depleted cells. Dephosphorylation, loss of hormone binding, Hsp90 dissociation and ATP-dependent cycling. J. Biol. Chem. **269:** 6571–6577.
39. **Jackson, D. A., and P. R. Cook.** 1985. Transcription occurs at the nucleoskeleton. EMBO J. **4:**919–925.
40. **Jackson, D. A., A. B. Hassan, R. J. Errington, and P. R. Cook.** 1993. Visualization of focal sites of transcription within human nuclei. EMBO J. **12:** 1059–1065.

41. **Jimenez-Garcia, L. F., and D. L. Spector.** 1993. *In vivo* evidence that transcription and splicing are coordinated by a recruiting mechanism. Cell **73:** 47–59.
42. **Kaufmann, S. H., S. Okret, A. C. Wikström, J.-A. Gustafsson, and J. H. Shaper.** 1986. Binding of the glucocorticoid receptor to the rat liver nuclear matrix. J. Biol. Chem. **261:**11962–11967.
43. **Khanna-Gupta, A., and V. C. Ware.** 1989. Nucleocytoplasmic transport of ribosomes in a eukaryotic system: is there a facilitated transport process? Proc. Natl. Acad. Sci. USA **86:**1791–1795.
44. **Lanford, R. E., and J. E. Butel.** 1979. Antigen relationship of SV40 early proteins to purified large T polypeptide. Virology **97:**295–306.
45. **Madan, A. P., and D. B. DeFranco.** 1993. Bidirectional transport of glucocorticoid receptors across the nuclear envelope. Proc. Natl. Acad. Sci. USA **90:** 3588–3592.
46. **Mancini, M. A., B. Shan, J. A. Nickerson, S. Penman, and W.-H. Lee.** 1994. The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. Proc. Natl. Acad. Sci. USA **91:**418–422.
47. **Mandell, R. B., and C. M. Feldherr.** 1990. Identification of two HSP70-related *Xenopus* oocyte proteins that are capable of recycling across the nuclear envelope. J. Cell Biol. **111:**1775–1783.
48. **Melchior, F., and L. Gerace.** 1995. Mechanisms of nuclear protein import. Curr. Opin. Cell Biol. **7:**310–318.
49. **Mendel, D. B., J. E. Bodwell, and A. Munck.** 1986. Glucocorticoid receptors lacking hormone-binding activity are bound in nuclei of ATP-depleted cells. Nature (London) **324:**478–480.
50. **Metzger, D. A., and K. S. Korach.** 1990. Cell-free interaction of the estrogen receptor with the mouse uterine nuclear matrix-evidence of saturability, specificity, and resistance to KCl extraction. Endocrinology **126:**2190–2195.
51. **Meyer, B. E., and M. H. Malim.** 1994. The HIV-1 Rev *trans*-activator shuttles between the nucleus and the cytoplasm. Genes Dev. **8:**1538–1547.
52. **Mittnacht, S., J. A. Lees, D. Desai, E. Harlow, D. O. Morgan, and R. A. Weinberg.** 1994. Distinct sub-populations of the retinoblastoma protein show a distinct pattern of phosphorylation. EMBO J. **13:**118–127.
53. **Mittnacht, S., and R. A. Weinberg.** 1991. G1/S phosphorylation of the retinoblastoma protein is associated with an altered affinity for the nuclear compartment. Cell **65:**381–393.
54. **Moroianu, J., and G. Blobel.** 1995. Protein export from the nucleus requires the GTPase Ran and GTP hydrolysis. Proc. Natl. Acad. Sci. USA **92:**4318–4322.
55. **Newmeyer, D. D., and D. J. Forbes.** 1988. Nuclear import can be separated into distinct steps *in vitro*: nuclear pore binding and translocation. Cell **52:** 641–653.
56. **Nickerson, J. A., and S. Penman.** 1992. The nuclear matrix: structure and involvement in gene expression, p. 343–380. *In* G. Stein and J. Lian (ed.), Molecular and cellular approaches to the control of proliferation and differentiation. Academic Press, Inc., San Diego, Calif.
57. **Orti, E., D. B. Mendel, L. I. Smith, J. E. Bodwell, and A. Munck.** 1989. A dynamic model of glucocorticoid receptor phosphorylation and recycling in intact cells. J. Steroid Biochem. **34:**85–96.
58. **Picard, D., and K. R. Yamamoto.** 1987. Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. EMBO J. **6:** 3333–3340.
59. **Pinol-Roma, S., and G. Dreyfuss.** 1992. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature (London) **355:**730–732.
60. **Qi, M., B. J. Hamilton, and D. DeFranco.** 1989. *v-mos* oncoproteins affect the nuclear retention and reutilization of glucocorticoid receptors. Mol. Endocrinol. **3:**1279–1288.
61. **Richardson, W. D., A. D. Mills, S. M. Dilworth, R. A. Laskey, and C. Dingwall.** 1988. Nuclear protein migration involves two steps: rapid binding at the nuclear envelope followed by slower translocation through nuclear pores. Cell **52:**655–664.
62. **Robinson, S. I., B. D. Nelkin, and B. Vogelstein.** 1982. The ovalbumin gene is associated with the nuclear matrix of chicken oviduct cells. Cell **28:**99–106.
63. **Rusconi, S., and K. R. Yamamoto.** 1987. Functional dissection of the hormone and DNA binding activities of the glucocorticoid receptor protein. EMBO J. **6:**1309–1315.
64. **Sanchez, E. R., M. Hirst, L. C. Scherrer, H. Y. Tang, M. J. Welsh, J. M. Harmon, S. S. J. Simons, G. M. Ringold, and W. B. Pratt.** 1990. Hormone-free mouse glucocorticoid receptors overexpressed in Chinese hamster ovary cells are localized to the nucleus and are associated with both hsp70 and hsp90. J. Biol. Chem. **265:**20123–20130.
65. **Schmidt-Zachmann, M. S., C. Dargemont, L. C. Kuhn, and E. A. Nigg.** 1993. Nuclear export of proteins: the role of nuclear retention. Cell **74:**493–504.
66. **Schuchard, M., M. Subramaniam, T. Ruesink, and T. C. Spelsberg.** 1991. Nuclear matrix localization and specific matrix DNA binding by receptor binding factor-1 of the avian progesterone receptor. Biochemistry **30:**9516–9522.
67. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under the control of the SV40 early region promoter. J. Mol. Appl. Genet. **1:**327–341.
68. **Spector, D. L.** 1993. Macromolecular domains within the cell nucleus. Annu. Rev. Cell Biol. **9:**265–315.
69. **Staufeniel, M., and W. Deppert.** 1983. Different structural systems of the nucleus are targets for SV40 large T antigen. Cell **33:**173–181.
70. **Stein, G. S., A. J. van Wijnen, J. L. Stein, J. B. Lian, J. P. Bidwell, and M. Montecino.** 1994. Nuclear architecture supports integration of physiological regulatory signals for transcription of cell growth and tissue-specific genes during osteoblast differentiation. J. Cell. Biochem. **55:**4–15.
71. **Stief, A., D. M. Winter, W. H. Stratling, and A. E. Sippel.** 1989. A nuclear DNA attachment site mediates elevated and position-independent gene activity. Nature (London) **341:**343–345.
72. **Sullivan, W. P., T. G. Beito, J. Proper, C. J. Krco, and D. O. Toft.** 1986. Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology **119:**1549–1557.
73. **Sun, J.-M., H. Y. Chen, and J. R. Davie.** 1994. Nuclear factor 1 is a component of the nuclear matrix. J. Cell. Biochem. **55:**252–263.
74. **van Steensel, B., G. Jenster, K. Damm, A. O. Brinkmann, and R. van Driel.** 1995. Domains of the human androgen receptor and glucocorticoid receptor involved in binding to the nuclear matrix. J. Cell. Biochem. **57:**465–478.
75. **van Steensel, B., A. D. van Haarst, E. R. de Kloet, and R. van Driel.** 1991. Binding of corticosteroid receptors to rat hippocampus nuclear matrix. FEBS Lett. **292:**229–231.
76. **Van Wijnen, A. J., J. P. Bidwell, E. G. Fey, S. Penman, J. B. Lian, J. L. Stein, and G. S. Stein.** 1993. Nuclear matrix association of multiple sequence-specific DNA binding activities related to SP1, ATF, CCAAT, C/EBP, OCT-1, and AP-1. Biochemistry **32:**8397–8402.
77. **Vassetzky, Y. S., C. V. De Moura Gallo, A. N. Bogdanova, S. V. Razin, and K. Scherrer.** 1993. The sequence-specific nuclear matrix binding factor F6 is a chicken GATA-like protein. Mol. Gen. Genet. **238:**309–314.
78. **Wansink, D. G., W. Schul, I. van der Kraan, B. van Steensel, R. van Driel, and L. de Jong.** 1993. Fluorescent labeling of nascent RNA reveals transcription by RNA polymerase II in domains scattered throughout the nucleus. J. Cell Biol. **122:**283–293.
79. **Wen, W., A. T. Harootunian, S. R. Adams, J. Feramisco, R. Y. Tsien, J. L. Meinkoth, and S. S. Taylor.** 1994. Heat-stable inhibitors of cAMP-dependent protein kinase carry a nuclear export signal. J. Biol. Chem. **269:**32214–32220.
80. **Wikstrom, A.-C., O. Bakke, S. Okret, M. Bronnegard, and J.-A. Gustafsson.** 1987. Intracellular localization of the glucocorticoid receptor: evidence for cytoplasmic and nuclear localization. Endocrinology **120:**1232–1242.
81. **Xing, Y., and J.-B. Lawrence.** 1991. Preservation of specific RNA distribution within the chromatin-depleted nuclear substructure demonstrated by in situ hybridization coupled with biochemical fractionation. J. Cell Biol. **112:**1055–1063.
82. **Xu, M., R. E. Hammer, V. C. Blasquez, S. L. Jones, and W. T. Garrard.** 1989. Immunoglobulin kappa gene expression after stable integration. II. Role of intronic mar and enhancer in transgenic mice. J. Biol. Chem. **264:**21190–21195.
83. **Yamamoto, K. R.** 1985. Steroid receptor regulated transcription of genes and gene networks. Annu. Rev. Genet. **19:**209–252.
84. **Yamamoto, K. R., and B. Alberts.** 1975. The interaction of estradiol-receptor protein with the genome: an argument for the existence of undetected specific sites. Cell **4:**301–310.
85. **Yamamoto, K. R., and B. M. Alberts.** 1976. Steroid receptors: elements for modulation of eukaryotic transcription. Annu. Rev. Biochem. **45:**721–746.
86. **Yang, J., and D. B. DeFranco.** 1994. Differential roles of heat shock protein 70 in the in vitro nuclear import of glucocorticoid receptor and simian virus 40 large tumor antigen. Mol. Cell. Biol. **14:**5088–5098.
87. **Zeitlin, S., A. Parent, S. Silverstein, and A. Efstratiadis.** 1987. Pre-mRNA splicing and the nuclear matrix. Mol. Cell. Biol. **7:**111–120.
88. **Zhang, G., K. L. Taneja, R. H. Singer, and M. R. Green.** 1995. Localization of pre-mRNA splicing in mammalian nuclei. Nature (London) **372:**809–812.