It has been shown previously that glucocorticoid receptors (GRs) that have undergone hormone-dependent translocation to the nucleus and have subsequently exited the nucleus upon hormone withdrawal are unable to recycle into the nucleus if cells are treated during hormone withdrawal with okadaic acid, a cell-permeable inhibitor of certain serine/threonine protein phosphatases. Using a green fluorescent protein (GFP) GR chimera (GFP-GR), we report here that okadaic acid inhibition of steroid-dependent receptor recycling to the nucleus is abrogated in cells treated for 1 h with colcemid to eliminate microtubule networks prior to steroid addition. After withdrawal of colcemid, normal cytoskeletal architecture is restored and okadaic acid inhibition of steroid-dependent GFP-GR nuclear recycling is restored. When okadaic acid is present during hormone withdrawal, GR that is recycled to the cytoplasm becomes complexed with hsp90 and binds steroid, but it does not undergo the normal agonist-dependent dissociation from hsp90 upon retreatment with steroid. However, when the cytoskeleton is disrupted by colcemid, the GR in okadaic acid-treated cells recycles from the cytoplasm to the nucleus in an agonist-dependent manner without dissociating from hsp90. This suggests that under physiological conditions where the cytoskeleton is intact, a dephosphorylation event is required for loss of high affinity binding to hsp90 that is required for receptor translocation through the cytoplasm to the nucleus along cytoskeletal tracts.

Although targeted movement of certain signaling proteins such as the steroid receptors, signal transducers and activators of transcription, and mitogen-activated protein kinases through the cytoplasm to the nucleus is necessary for signal transduction, virtually nothing is known about the machinery that enables these proteins to traverse the cytoplasm. Steroid receptors undergo a constant shuttling into and out of the nucleus (1–4) (for review, see Ref. 5) and, depending upon the receptor and the cell type, the hormone-free, untransformed receptor may be predominantly nuclear or cytoplasmic in location. In most cells, the untransformed glucocorticoid receptor (GR)1 is predominantly localized in the cytoplasm, and upon hormone binding and transformation, the receptor relocates to the nucleus (6–8) in a manner that is determined by nuclear localization signal (NLS) sequences in the receptor (6). Because movement of the receptor can be initiated by addition of steroid, the GR is a particularly useful model for studying targeted protein movement through the cytoplasm.

Studies performed with inhibitors suggest that the protein chaperone hsp90 and an okadaic acid-sensitive protein phosphatase may each play a role in GR translocation from the cytoplasm to the nucleus. The involvement of hsp90 in receptor movement is likely to involve dynamic assembly and disassembly of GR-hsp90 heterocomplexes. For example, Yang and DeFranco (9) showed that molybdate, which binds to hsp90 and stabilizes GR-hsp90 heterocomplexes in vivo (10), trapped the GR in the cytoplasm of cells chronically exposed to hormone. It was suggested that the receptors can be exported from nuclei after hormone-dependent translocation, but they cannot be reimported into nuclei in the presence of molybdate (10). Association of receptors with hsp90 is a dynamic process (11), and it has been shown that GR and hsp90 can move together from the cytoplasm to the nucleus (12). It has also been shown that treatment of cells with geldanamycin, an antibiotic that binds to hsp90 (13) at its nucleotide binding site (14) and prevents formation of normal receptor-hsp90 heterocomplexes (15), impedes steroid-induced movement of the GR from the cytoplasm to the nucleus (16). Taken together, these observations are consistent with the notion that hsp90 plays some role in GR movement.

The notion that a phosphatase may be required for receptor shuttling evolved from the work of Qi et al. (7, 17), who found that GRs in cells transformed with v-Mos, an oncoprotein that is a cytoplasmic serine/threonine protein kinase, undergo hormone-dependent transfer to the nucleus but that they are inefficiently retained, and they cycle back to the cytoplasm, where they do not regain the capacity for hormone-dependent translocation. Subsequently, DeFranco et al. (18) showed that okadaic acid, a serine/threonine protein phosphatase inhibitor, acts like v-Mos to trap GR that has cycled out of the nucleus into the cytoplasm in a form that cannot undergo hormone-dependent recycling to the nucleus. These observations suggest that a cytoplasmic phosphatase is required for recycling of GR that has passed through stages of nuclear import, transcriptional activation, and nuclear export (18). It is not known whether the phosphatase activity is required for GR transformation to a state that can be translocated or whether it is required for subsequent movement of the transformed receptor (18).

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‡ The abbreviations used are: GR, glucocorticoid receptor; NLS, nuclear localization signal; DMEM, Dulbecco's modified Eagle's medium; hsp, heat shock protein; GFP, green fluorescent protein; CORT, corticosterone; OA, okadaic acid; TES, 2-(2-hydroxyethyl)-1,1-bis-(hydroxymethyl)ethyl]amino)ethanesulfonic acid.

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recently, we have used cytoskeletal disrupting agents, such as colcemid and cytochalasin D, and a fusion protein of murine GR with Aequorea green fluorescent protein (GFP-GR) to determine whether there is any linkage between hsp90-dependent receptor movement and the cytoskeleton (19). As previously reported by Perrot-Applanat et al. (20), for the progesterone receptor, we found that GFP-GR underwent rapid (t_{1/2} ≈ 5 min) steroid-dependent translocation to the nucleus both in cells with intact cytoskeleton and in cells with completely disrupted cytoskeletal networks. However, in cells with a normal cytoskeleton, the hsp90 inhibitor geldanamycin slowed translocation of the GFP-GR by close to an order of magnitude (t_{1/2} ≈ 45 min), whereas in cells with disrupted cytoskeletal networks, geldanamycin had no effect on the translocation rate (t_{1/2} ≈ 5 min). This suggests two mechanisms of GR movement. Under physiological conditions where the cytoskeleton is intact, diffusion is limited, and the GFP-GR utilizes a movement machinery in which the activity of hsp90 plays a role. In cells where the cytoskeletal networks have been artifactualy disrupted, movement is still steroid-dependent, but the transformed GFP-GR moves through the cytoplasm by diffusion and is not affected by geldanamycin.

In this work, we show that treatment of cells with okadaic acid inhibits GR transformation as defined by loss of its high affinity association with hsp90, and we show that disruption of microtubules with colcemid eliminates the okadaic acid block of hormone-dependent recycling of GFP-GR to the nucleus. We suggest that under physiological conditions where the cytoskeleton is intact, the GR shuttles between the cytoplasm and the nucleus with the help of a machinery that utilizes cytoskeletal tracts, involves the dynamic activity of hsp90, and requires the activity of an okadaic acid-sensitive phosphatase. However, when the cytoskeleton is disrupted, the GR moves through the cytoplasm by diffusion in a manner that is still steroid-dependent but does not require the okadaic acid-sensitive phosphatase activity and hsp90 dissociation.

**EXPERIMENTAL PROCEDURES**

**Materials**

NIH/3T3 mouse embryonic fibroblasts were purchased from the American Type Culture Collection (Rockville, MD). Phenol red-free Dulbecco’s modified Eagle’s medium (DMEM) and LipofectAMINE were from Life Technologies, Inc. Geldanamycin was obtained from the Drug Synthesis and Chemistry branch of the Developmental Therapeutics Program, National Cancer Institute. Colcemid, colchicine, γ-lumicolchicine, and charcoal-stripped, delipidated calf serum were from Sigma. BuGR2 monoclonal anti-glucocorticoid receptor IgG was from Affinity BioReagents (Golden, CO), and the AC88 monoclonal IgG anti-hsp90 was from StressGen (Victoria, BC, Canada). Hybridoma cells producing the PGR monoclonal IgG anti-GR (21) were generously provided by Jack Bodwell (Dartmouth Medical School). Construction of the GFP-GR expression plasmid was described previously (19).

**Methods**

**Cell Culture and Transfection—**NIH/3T3 cells were grown on 22 × 22-mm coverslips in DMEM supplemented with 10% bovine calf serum in 35-mm tissue culture dishes. When cells were ~60% confluent, they were rinsed three times with serum-free medium and then incubated for an additional hour in fresh medium. For each transfection of GFP-GR cDNA, a solution containing 2.5 μg of DNA and 10 μl of LipofectAMINE in 0.8 ml of Opti-MEM I medium was added to culture dishes containing 2 ml of DMEM and mixed gently to ensure uniform distribution. Cells were incubated with the transfection mixture for 6 h at 37 °C, and the medium was then replaced by complete growth medium for an additional 18 h of incubation. At the end of this incubation, the coverslips were washed extensively with and then incubated overnight in phenol red-free DMEM supplemented with 10% charcoal-stripped, delipidated bovine calf serum. The cells were used for GFP-GR translocation experiments.

**GFP-GR Translocation—**For receptor translocation and steroid withdrawal experiments, cells transfected as described above were incubated for 1 h with 0.1 μM corticosterone to permit GFP-GR translocation to the nucleus. The medium was then removed and cells were washed 3–4 times and incubated for 20 h in phenol red-free DMEM with charcoal-stripped serum and with or without 50 nM okadaic acid. After 20 h, 0.1 μM corticosterone was readded for 1 h to permit GFP-GR recycling into the nucleus. For microtubule disruption, 0.6 μg/ml colcemid was added 1 h prior to readdition of corticosterone. For geldanamycin inhibi-
Fluorescence Visualization—At the end of the 1-h incubation with corticosterone, the coverslips were rinsed with phosphate-buffered saline at room temperature and simultaneously fixed and permeabilized by immersion in cold methanol (−25 °C) for 30 min. Cells were rinsed again with phosphate-buffered saline, and the coverslips were inverted onto a slide with 5 μl of mounting solution (1 mg/ml p-phenylenediamine in 10% phosphate-buffered saline, 90% glycerol, pH 9.0). Cells were photographed with a Leitz Aristoplan epillumination microscope and a Leitz Vario-Orthomat camera using T-Max 3200 film. The bar in Fig. 1 represents 10 μm.

Scoring of GFP-GR Translocation—Cells were scored for GFP-GR translocation as we have described previously (19), using a score of 4 for nuclear fluorescence much greater than cytoplasmic fluorescence, 3 for nuclear fluorescence greater than cytoplasmic fluorescence, 2 for nuclear fluorescence equal to cytoplasmic fluorescence, 1 for nuclear fluorescence less than cytoplasmic fluorescence, and 0 for nuclear fluorescence equal to cytoplasmic fluorescence. The translocation scores represent the mean ± S.E. from three experiments in which >100 cells per condition per experiment were scored. Significance of differences was assessed by analysis of variance followed by a Bonferroni t test.

Assay of GR-associated hsp90—Untransfected 3T3 cells treated with corticosterone and okadaic acid, as indicated in Fig. 6, were suspended in 1.5 volumes of HEM buffer (10 mM HEPES (pH 7.4), 1 mM EDTA, 20 mM sodium molybdate) and ruptured by Dounce homogenization. Cell homogenates were centrifuged for 30 min at 100,000 × g, with the supernatant being the cytosol. The GR was immunoadsorbed from replicate aliquots (500 μl) of cytosol with FiGR ascites as described previously (16). The immune pellets were washed 4 times by suspension in 1 ml of TEGM buffer (10 mM TES, 2-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]ethyl]amino)ethanesulfonic acid (pH 7.6), 50 mM NaCl, 4 mM EDTA, 10% glycerol, 20 mM molybdate), and boiled in SDS sample buffer with 10% β-mercaptoethanol, and proteins were resolved on 8% SDS-polyacrylamide gels. After transfer to Immobilon-P membranes, the GR was probed with 2 μg/ml BuGR and hsp90 with 1 μg/ml AC88, and the immunoblots were incubated a second time with 125I-labeled goat anti-mouse IgG.

RESULTS

Okadaic Acid Inhibition of GFP-GR Recycling from Cytoplasm to Nucleus—To determine whether okadaic acid blocks agonist-dependent recycling of the GFP-GR in the same manner as previously reported for endogenous GR in rat fibroblasts (18), we performed the experiments summarized in the legend to Fig. 1. In 3T3 cells expressing GFP-GR, the fusion protein is localized to the cytoplasm in the absence of steroid (Fig. 1A) and is translocated to the nucleus when cells are incubated with the agonist corticosterone (Fig. 1C). The same agonist-dependent translocation is seen in cells incubated simultaneously with okadaic acid (Fig. 1B and D). Under both conditions (i.e. with and without okadaic acid), withdrawal of corticosterone for 20 h is accompanied by return of the GFP-GR to the cytoplasm (Fig. 1, E and F). In the absence of okadaic acid, retreatment with corticosterone causes recycling of the GFP-GR to the nucleus (Fig. 1G). However, in the presence of okadaic acid, agonist-dependent recycling to the nucleus is inhibited (Fig. 1H).

Fig. 2A shows the time course of the return of GFP-GR to the cytoplasm upon withdrawal of corticosterone. In all of the experiments in this work, we use a 20-h withdrawal period before retreatment with agonist. Fig. 2B presents the concentration dependence of okadaic acid inhibition of agonist-dependent GFP-GR recycling to the nucleus. In this case, cells were treated with corticosterone to translocate the GFP-GR to the nucleus and then withdrawn in the presence of various concentrations of okadaic acid. At the end of the 20-h withdrawal period, corticosterone was added to each culture, and the incubations were continued for an additional 1 h to permit the GFP-GR to reenter the nucleus. At concentrations below 25 nM okadaic acid, complete recycling to the nucleus is achieved, but at higher concentrations, increasing inhibition is observed. In the rest of the experiments in this work, we use 50 nM okadaic acid, which is the minimum concentration required for maximum inhibition of GFP-GR recycling to the nucleus.

Cytoskeletal Disruption with Colcemid Restores Agonist-induced GFP-GR Recycling in Okadaic Acid-treated Cells—To determine the effect of cytoskeletal disruption on okadaic acid inhibition of GFP-GR recycling, corticosterone-treated cells were withdrawn in the presence or absence of okadaic acid and then treated for 1 h with colcemid. We have shown previously that a 1-h treatment with 0.6 μg/ml colcemid completely eliminates microtubules in 3T3 cells (19). As shown in Fig. 3, the GFP-GR is predominantly cytoplasmic in cells that have been withdrawn in the presence of okadaic acid and then treated with colcemid (Fig. 3B, upper right). However, when these cells with disrupted cytoskeleton are treated again with corticosterone, there is complete translocation of the GFP-GR to the nucleus, even in the presence of okadaic acid (Fig. 3B, lower right). In control cells that were treated identically but not exposed to colcemid, GFP-GR translocation to the nucleus was inhibited by okadaic acid (Fig. 3A, lower right). Thus, the colcemid treatment restored agonist-induced GFP-GR recycling to okadaic acid-treated cells.

The effect of cytoskeletal disruption on the rate of agonist-induced nuclear reentry of the GFP-GR is shown in Fig. 4. Reentry occurs at a similar rate (t1/2 ~5 min) in okadaic acid-free cells with disrupted cytoskeleton (open circles) as in cells with an intact cytoskeletal system (closed circles). In cells withdrawn in the presence of okadaic acid and not treated with colcemid (closed squares), about one-third of the cytoplasmic GFP-GRs move to the nucleus within the first few minutes.
After readdition of corticosterone, but a plateau of movement is achieved, and the remainder of the GFP-GRs stays in the cytoplasm. The GFP-GR that moves to the nucleus under this condition may be GFP-GR that was synthesized during the withdrawal period, and because it has not cycled through the nucleus, it is not affected by okadaic acid, as shown in Fig. 1D. In contrast, the GFP-GRs in okadaic acid-treated cells where the cytoskeleton has been disrupted by colcemid (open squares) undergo essentially complete agonist-induced nuclear reentry at the same rapid rate as in cells not treated with okadaic acid. The Effects of Both Okadaic Acid and Cytoskeletal Disruption Are Reversible—In Fig. 5A, cells withdrawn in the presence of okadaic acid (condition 3) were either treated with corticosterone (condition 4) or washed and incubated for 1 h in okadaic acid-free medium prior to treatment with corticosterone (condition 5). It is clear that the okadaic acid inhibition of GFP-GR reentry shown in condition 4 is eliminated when cells are washed free of okadaic acid, as shown in condition 5. As shown in condition 6, the agonist-dependent nuclear reentry of about one-third of the cytoplasmic GFP-GRs that occurs in okadaic acid-treated cells (cf. condition 4 with 3) is blocked if the hsp90 inhibitor geldanamycin is present during the 20-min incubation with corticosterone. Like the inhibition of reentry with okadaic acid alone, the total inhibition of reentry achieved by geldanamycin and okadaic acid together is reversed by washing the cells (condition 7).

We have shown previously that the cytoskeletal network returns to normal when 3T3 cells with completely disrupted microtubules are incubated for a short time in colcemid-free medium (19). Fig. 5B shows that incubation of 3T3 cells in colcemid-free medium restores okadaic acid inhibition of GFP-GR nuclear reentry to colcemid-treated cells (cf. condition 5 with 4). The proposal that it is disruption of the cytoskeleton that permits the GFP-GR in colcemid-treated cells to travel through the cytoplasm to the nucleus despite the action of okadaic acid is supported by the data of conditions 6 and 7 in Fig. 5B. In this case, the 3T3 cells that were withdrawn in the presence of okadaic acid were incubated for 1 h either with colchicine or with \(\gamma\)-lumicolchicine, a biologically inactive isomer of colchicine that does not interact with tubulin (22). We have previously reported that 3T3 cells treated with 1 \(\mu\)M colchicine lose their microtubules, whereas cells treated with 1 \(\mu\)M \(\gamma\)-lumicolchicine do not (19). As shown in Fig. 5B, okadaic acid did not inhibit GFP-GR nuclear reentry in colchicine-treated cells (condition 6), but it inhibited reentry in \(\gamma\)-lumicolchicine-treated cells (condition 7).

In Okadaic Acid-treated Cells, the GR Does Not Undergo Agonist-dependent Dissociation from hsp90—The GR is recovered from hormone-free cells as a 9 S GR:hsp90 heterocomplex, and shortly after treatment of cells with steroid, it is recovered from cytosol as the 4 S hsp90-free GR (for review, see Ref. 23). This steroid-dependent transformation of the receptor has been regarded as a prerequisite to translocation of the GR from the cytoplasm to the nucleus. In the experiment corresponding to Fig. 6A, we asked whether okadaic acid blocked receptor transformation. In this case, uninfected 3T3 cells were treated with corticosterone and okadaic acid as above, the cells were then ruptured, and the endogenous 3T3 cell GR was immunoadsorbed and assayed for receptor-associated hsp90 by West-
GR transfers to the nucleus within 10–15 min in colcemid-treated cells (8). Thus, we asked whether receptors that were recycled into the nucleus in cells that were withdrawn in the presence of okadaic acid and colcemid were recovered in the hsp90-free or hsp90-bound form. The 15-min interval after steroid treatment was chosen for the experiments corresponding to Fig. 6, because the GR that has transferred to the nucleus is still recovered in the cytosolic fraction after cell rupture. After 20 min of steroid treatment, most of the GR is retained in the nuclear fraction of colcemid-treated cells (data not shown). As shown in Fig. 6B, receptors that have been withdrawn in either the presence or absence of okadaic acid and then treated with colcemid are in heterocomplexes with hsp90. Corticosterone treatment of cells with a disrupted cytoskeleton that were withdrawn in the absence of okadaic acid resulted in hsp90-free GR, but in cells withdrawn in the presence of okadaic acid, receptors remain in heterocomplex with hsp90. Thus, the receptors in okadaic acid-withdrawn cells where the cytoskeleton is disrupted undergo agonist-dependent translocation to the nucleus (Fig. 3B) without undergoing transformation as defined by agonist-dependent dissociation of the GR-hsp90 heterocomplex (Fig. 6B).

**DISCUSSION**

Two mechanistically different inhibitors of steroid-dependent GR cytoplasmic nuclear translocation have been identified as geldanamycin and okadaic acid. Geldanamycin markedly inhibits the rate of receptor translocation, but in time, all of the receptors eventually become localized in the nucleus (16, 19). Geldanamycin inhibits both the initial transfer of the GR (16, 19) as well as the recycling of GR that has exited the nucleus upon withdrawal of hormone (Fig. 5A and data not shown). Because geldanamycin is an hsp90 inhibitor, we have suggested that dynamic assembly of steroid-bound GR heterocomplexes with hsp90 and its associated immunophils, such as FK506 binding protein 52, by the hsp90-based chaperone system plays a role in GR movement through the cytoplasm. Because geldanamycin slows but does not block GR movement,
it seems that the role of the hsp90-based chaperone system is to facilitate movement. Perhaps it does so by facilitating the association of the receptor with a piece of movement machinery with which the receptor associates less efficiently when assembly of mature GR-hsp90 heterocomplexes is inhibited by geldanamycin.

In contrast to geldanamycin, okadaic acid does not inhibit the initial steroid-dependent transfer of the GR from cytoplasm to nucleus; rather, it inhibits only the recycling of receptors that have passed through the original cycle of nuclear import, transcriptional activation, and nuclear export (18) (Fig. 1). Also, in contrast to geldanamycin’s inhibition of the rate of movement, okadaic acid blocks recycling of the majority of receptors (Fig. 4). Okadaic acid appears to block receptor recycling by inhibiting a dephosphorylation event that is required for steroid-dependent receptor transformation (Fig. 6). The GR is a phosphoprotein (for review, see Ref. 24) that becomes hyperphosphorylated after cells are exposed to agonist (25, 26). Dephosphorylation of the hyperphosphorylated GR itself could be the event required for receptor transformation, but other components of the untransformed receptor complex are also phosphoproteins (e.g. hsp90 and FK506 binding protein 52), and their dephosphorylation or the dephosphorylation of another protein may be critical for receptor transformation in intact cells.

Here we have assayed receptor transformation by assaying the decrease in the fraction of receptors recovered as GR-hsp90 heterocomplexes from steroid-withdrawn cells that were retreated with corticosterone (Fig. 6). This steroid-dependent transformation of the receptor to a form that is no longer in stable heterocomplex with hsp90 is apparently required to permit the GR to associate with the movement machinery in normal cells with intact cytoskeleton. In contrast to hormone-free progesterone receptor-hsp90 or estrogen receptor-hsp90 heterocomplexes, which are constitutively localized to the nucleus (for review, see Ref. 23), both of the GR NLSs appear to be blocked or conformationally inactive when the unliganded GR is in stable heterocomplex with hsp90. This may explain why the unliganded GR is cytoplasmic. Consistent with the notion that the NLS is activated upon GR transformation, it has been shown that dissociation of GR from stable heterocomplex with hsp90 permits access of an NLS-specific antibody to the NLS (27, 28). In cells that have been withdrawn in the presence of okadaic acid, the GR is in stable heterocomplex with hsp90 (Fig. 6), and it binds steroid normally (18), but receptor transformation as assayed by conversion to hsp90-free GR is blocked (Fig. 6).

Several years ago, it was shown that microtubule disruption does not prevent cytoplasmic nuclear translocation of the progesterone receptor (20) or the GR (8), and it does not affect steroid-mediated transcriptional activation by the GR (29). In cells treated with colcemid, neither geldanamycin (19) nor okadaic acid (Figs. 3–5) inhibits cytoplasmic nuclear translocation of the GR. Thus, when the cytoskeleton is disrupted, the GR appears to move by a different mechanism. A reasonable model for the normal cell would be that, like vesicles, the GR moves through the cytoplasm on cytoskeletal tracts requiring the participation of cytoskeleton-associated motor proteins. When the cytoskeletal network is intact, diffusion of protein solutes like the GR is limited, and in some way dynamic cycling of the liganded, transformed GR with hsp90 facilitates a connection between the NLS1 and the movement system. In contrast, the comparatively stable association of the unliganded, untransformed GR with hsp90 blocks access of NLS recognition pro-tein(s) to NL1. Okadaic acid appears to block receptor transformation, which is required for movement along the cytoskeleton-based movement system. We speculate that when the cytoskeleton is disrupted, a major limitation on diffusion of protein solutes has been removed, and the GR may diffuse through the cytoplasm, with its penetration through the nuclear pores being determined by interactions with nuclear uptake proteins such as importin (for review of the nuclear import of steroid receptors, see Ref. 5). It is interesting that recycling of the GR in okadaic acid-withdrawn cells treated with colcemid is strictly steroid-dependent (Figs. 3B and 5B). Yet, under these conditions, the GR appears to remain in stable association with hsp90 (Fig. 6B). Some steroid-dependent event other than dissociation from hsp90 must have occurred such that GR transformation is uncoupled from GR movement in the cell treated with both colcemid and okadaic acid.

Because Perrot-Applanat et al. (20) demonstrated that the progesterone receptor could move from the cytoplasm to the nucleus in cells where the cytoskeletal networks were completely disrupted, it has been assumed that the cytoskeleton is not involved in steroid receptor movement through the cytoplasm of the physiologically normal cell. The observations represented in this paper and those of Galigniana et al. (19) suggest that there are two movement mechanisms and that intact cytoskeleton is required for GR movement in the physiologically normal cell.

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