# Control of Steroid Receptor Function and Cytoplasmic-Nuclear Transport by Heat Shock Proteins

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## Summary

**As targeted proteins that move within the cell, the steroid receptors have become very useful probes for understanding the linked phenomena of protein folding and transport. From the study of steroid receptor-associated proteins it has become clear over the past two years that these receptors are bound to a multiprotein complex containing at least two heat shock proteins, hsp90 and hsp56. Attachment of receptors to this complex in a cell-free system appears to require the protein unfolding/folding activity of a third heat shock protein, hsp70. Like the oncogenic tyrosine kinase pp60*src*, steroid receptors bind to this complex of chaperone proteins at the time of their translation. Binding of the receptor to the hsp90 component of the system occurs through the hormone binding domain and is under strict hormonal control. The hormone binding domain of the receptor acts as a transferable *regulatory unit* that confers both tight hormonal control and hsp90 binding onto chimaeric proteins. The model of folding and transport being developed for steroid receptors leads to some general suggestions regarding the folding and transport of targeted proteins in the cell.**


## Introduction

Proteins functioning as transcription factors that regulate expression from a limited number of genes must be transported to and within the nucleus in a precisely targeted manner. The steroid receptors are transcription factors bearing nuclear localization signals whose cellular location and transcriptional activating activity are under tight hormonal control. This regulation is exerted by the receptor hormone binding domain. As originally shown by Picard and Yamamoto[1,2] the hormone binding domain of a steroid receptor can be fused to proteins of very different structure, bringing both their intracellular transport and transcriptional activating activity under hormonal control. This ability of the hormone binding domain both to regulate receptor activity and to function as a *regulatory unit* that can confer hormone regulation on the function of structurally different proteins can be explained through the hormone-regulated binding of this receptor domain to an abundant heat shock protein, hsp90.

The central speculation of this review will be that hsp90 is a component of a structure containing several proteins involved in protein folding and that steroid receptors and other molecules remain attached to this structure (a transportosome) while undergoing targeted transport in the cell. During evolution, the steroid receptors have brought their attachment to this elemental and conserved protein folding/transport system under hormonal control.

## Receptor Transformation and hsp90 Binding

The early studies of steroid receptor structure demonstrated that receptors could be recovered from hormone-free cells in two forms as defined by their behavior on sucrose gradient centrifugation. The small 4S form in cytosol was found to be a monomer (or in some cases a homodimer) that bound with high affinity to DNA and was formed at the expense of a larger 9S form without DNA binding activity. If cells were exposed to hormone prior to rupture, then the receptors were recovered in tight association with the nuclear fraction, requiring salt and detergent for their extraction in the 4S state. Because of their tendency to dissociate into the 4S form, the 9S cytosolic complexes remained uncharacterized for almost 15 years. Some investigators regarded the 9S complex as a receptor tetramer and the possibility that it represented a physiologically meaningless protein aggregate was always a consideration. By the late 1970s, it had been established that cytosolic steroid receptors could be stabilized in their 9S, non-DNA-binding form by molybdate and some other transition metal oxyanions (see Pratt[3] for review). The 9S receptor was said to be in an untransformed state, and steroids were considered to promote the *transformation* of their receptors from the 9S form to the 4S, DNA-binding form, both in intact cells and in cytosols prepared from hormone-free cells.

In the early 1980s, several laboratories purified molybdate-stabilized progesterone and glucocorticoid receptors, and in each case the major protein that was purified was 90 kDa[3]. A significant advance was made when Radanyi *et al.*[4] prepared a monoclonal antibody to the 90 kDa protein. The 90 kDa protein was shown to be distinct from the steroid-binding proteins and Joab *et al.*[5] used the antibody to demonstrate that the 90 kDa protein was a common component of molybdate-stabilized progestin, estrogen, androgen and glucocorticoid receptor complexes. It rapidly became clear that the 90 kDa receptor-associated protein was an ubiquitous, abundant, cytoplasmic phosphoprotein, and in 1985, the groups of Baulieu, Toft and myself established that it was hsp90[6-8]. At the same time, Mendel *et al.*[9] demonstrated that steroid treatment promoted dissociation of the glucocorticoid receptor from hsp90 in intact cells, and subsequently our laboratory[10] and that of Gustafsson[11] demonstrated that binding of glucocorticoid to cytosolic receptors promotes both temperature-dependent dissociation of the receptor from hps90 and simultaneous conversion of the receptor to the DNA binding state. The literature establishing a relationship between the untransformed state of cytosolic steroid receptors and their binding to hsp90 has been reviewed in detail[3].

Two genetic observations argue strongly that binding of

the glucocorticoid receptor to hsp90 reflects a physiologically important protein-protein interaction in the intact cell. First, analysis of receptors produced after transfection of hormone-free cells with mutant human glucocorticoid receptor cDNAs demonstrated that steroid-inducible forms of the receptor were recovered in molybdate-stabilized cytosols entirely as 9S complexes, whereas mutant receptors with constitutive activity were recovered only in the dissociated 4S form[12]. Thus, the 9S, receptor-hsp90 complex is probably derived from the physiologically inactive state of the receptor that is activated by steroid in the cell, and the 4S, dissociated form is derived from receptor that is active in transcriptional enhancement. Recently, Picard *et al.*[13] analyzed the glucocorticoid response in a strain of *S. cerevisiae* that was modified to produce only 5% of the hsp90 present in wild-type cells. They showed that glucocorticoid receptors were activated with markedly reduced efficacy when hsp90 levels were low. This argues strongly for a role for hsp90 in the signal transduction pathway for steroid receptors.

## The 'Docking Complex'

During the 1980s, it became clear that all members of the steroid/thyroid receptor family did not behave in the same manner with regard to their localization in intact cells. In hormone-free cells, glucocorticoid receptors are usually located in the cytoplasm, and after addition of steroid, they are rapidly translocated to the nucleus. In contrast to the glucocorticoid receptor, unliganded estrogen and progesterone receptors apparently move directly from their cytoplasmic sites of synthesis into the nucleus where they remain in a loosely-bound, inactive docking state until bound by hormone (see Pratt[14] for review). These distinctions do not always apply. For example, mouse glucocorticoid receptors that are overexpressed in hormone-free CHO cells are located entirely within the nucleus[15]. Regardless of whether unliganded steroid receptors are localized in the nucleus or in the cytoplasm, after hypotonic cell rupture, they are recovered in the cytosol in a 9S complex with hsp90[14]. Thus, we were led to the concept that steroid receptors remain 'docked' to an hsp90-containing structure until binding of hormone triggers dissociation from hsp90, permitting progression of the receptor to high affinity nuclear binding sites where the primary events in transcriptional activation occur[14].

This concept of attachment of the receptor to a *docking* structure fits well with the observation that the receptor hormone binding domain is able to confer hormone regulation on proteins of different structure. From the results of a variety of experimental approaches, it is very clear that the hormone binding domains of glucocorticoid and progesterone receptors determine their interaction with hsp90[12,16-20]. It is also clear that glucocorticoid or progesterone binding to their receptors promotes the temperature-dependent dissociation of the receptors from hsp90[10,11,21-23]. Thus, it is reasonable to predict that when the hormone binding domain of a steroid receptor is fused to another protein, the fusion protein will be bound to the hsp90-containing docking complex until hormone binding promotes its release. In the usual case of the glucocorticoid receptor where the hormone binding domain contains a nuclear localization signal that is repressed when the receptor is associated with hsp90, a fusion protein containing the hormone binding domain would be localized in the cytoplasm in the hormone-free cell[1]. After steroid-mediated dissociation of hsp90, the fusion protein would be transported into the nucleus. This is what Picard and Yamamoto observed with fusion proteins prepared from β-galactosidase and portions of the glucocorticoid receptor[1]. We have assayed several of these fusion proteins for their association with hsp90, and we find in all cases where β-galactosidase movement in the cell is brought under hormonal control, the fusion protein is bound to hsp90 (Scherrer *et al.*, submitted manuscript). In those cases, such as the estrogen or progesterone receptors, where the nuclear localization signal is not blocked when the receptor is bound to hsp90, one might expect a fusion protein containing both the hormone binding domain and a nuclear localization signal to be transported to the nucleus. But, like the intact receptor itself, the fusion protein would remain docked in the nucleus until the hormone triggered its release and progression to its site of action.

The docking model allows us to account for a great deal of structural difference in the molecule that is being regulated by fusion to the hormone binding domain of a steroid receptor. It is difficult to accomodate the regulation of structurally different molecules in models that assume direct repression of the active sites of fusion proteins by hsp90. In the case of steroid receptors in their 9S heterocomplex form, it is clear that hsp90 directly represses DNA binding activity[24]. However, in the intact cell, it is likely that the docking function of hsp90 is the important effect with respect to inhibition of transcriptional activation. In a docking model, it is inherently unimportant whether the function to be controlled through fusion to the hormone binding domain is exerted in the nucleus, or at the inside of the plasma membrane, or elsewhere in the cytoplasm. If the protein that is fused undergoes transport to a targeted site of action, then in theory it could be regulated in such a fusion approach. This underlines the interest of a number of investigators in bringing the function of a variety of proteins under hormonal control by fusing them to the hormone binding domain of steroid receptors. One inherent problem to using the hormone binding domain of the glucocorticoid receptor is that it contains its own nuclear localization signal, which is unmasked by hormone and may be dominant[1]. In contrast, the nuclear localization signals of the estrogen receptor lie outside of its hormone binding domain, which clearly confers estrogen regulation onto fusion proteins[25]. In some cases, the estrogen receptor hormone binding domain may yield better control, at least when the protein to be brought under hormonal control is active at a nonnuclear site.

In contrast to steroid receptors, the evolutionarily more primitive members of the steroid/thyroid hormone receptor family, such as thyroid hormone and retinoic acid receptors, are transported directly from their cytoplasmic sites of synthesis into tight association with the nucleus in hormone-free cells[14]. It is thought that in their unliganded state, these receptors proceed directly to appropriate response elements

in the genome, and in some cases, they may produce transcriptional inhibition in the unliganded form. Consistent with this behavior, we have found that thyroid hormone[26] and retinoic acid[27] receptors do not bind to hsp90 (or they do not bind it with high enough affinity to permit easy detection of the complex).

## Composition of the Docking Complex

Although all of the initial interest in this field focused on steroid receptor-hsp90 interactions, by 1990, it had become clear that the docking complex contained additional proteins. Several lines of investigation have converged to permit a model of the docking structure as a complex of protein folding enzymes. This story is developing rapidly and it is not my goal to present all of the details here, as much of the information has already been reviewed[14]. Here, I will try to summarize and integrate the structural data on the docking complex and in a subsequent section I will review the recent work demonstrating its protein folding function.

When unliganded progesterone or glucocorticoid receptors are immunoadsorbed under the gentlest conditions designed to maintain as much of the structure of the native heteroprotein complex as possible, several proteins have been found to coimmunoadsorb with the receptors in addition to hsp90. For example, the Toft laboratory has identified proteins of 70, 54, 50 and 23 kDa in the avian progesterone receptor complex[22,23], and we have identified proteins of approximately 70, 56 and 23 kDa in the glucocorticoid receptor complex[15,28]. A cross-linking approach also suggests the existence of a ~14 kDa protein that can be recovered with the glucocorticoid receptor complex[29]. Two of these proteins are known to be heat shock proteins.

The 70 kDa progesterone receptor-associated protein was identified as a constitutive member of the hsp70 family[22,23]. Hsp70 is not associated with glucocorticoid receptor complexes recovered from mouse L cells[15] or WEHI-7 mouse lymphoma cells[30], but a substantial amount of hsp70 is associated with the mouse glucocorticoid receptor overexpressed in CHO cells[15]. This distinction may be important. As mentioned above, the untransformed mouse glucocorticoid receptor complex in CHO cells and progesterone receptor complexes in a variety of cell types are located in the cell nucleus but the L cell glucocorticoid receptor complex and probably also the WEHI-7 cell complex are located in the cytoplasm. The hsp70 family has a well known protein unfoldase activity and it is possible that nuclear entry or some subsequent event in receptor transport requires unfoldase activity. As described below, there is substantial reason to believe that the unfoldase activity of hsp70 may be required initially to constitute the steroid receptors into a complex with hsp90. Unlike hsp90, hsp70 does not dissociate when cytosolic steroid receptors are transformed[15,23] and it does not affect the DNA binding activity of the receptor[31]. Like hsp90, hsp70 appears to bind directly to the hormone binding domain of the receptor[18].

In various systems, the 56 kDa receptor-associated protein has been reported to be 53 to 59 kDa. We have found that the major form of the human protein is 56 kDa[32]. This protein was originally identified in the Faber laboratory when a monoclonal antibody (EC1) prepared against the untransformed rabbit progesterone receptor complex was shown to recognize a protein associated with progestin, estrogen, androgen and glucocorticoid receptor complexes[33]. When we used this antibody to immnuadsorb the 56 kDa protein from human IM-9 lymphocyte cytosol, we found that large amounts of both hsp90 and hsp70 were coimmunoadsorbed, suggesting that the three proteins were bound together in a higher order complex[32]. This finding was followed by the observation of Sanchez[34] that the 56 kDa receptor-associated protein is itself a heat shock protein and it is now designated hsp56.

Two recent papers have provided a great deal of information regarding the possible function of hsp56. Yem *et al.*[35] immobilized the potent immunosuppressant drug FK506 to Affi-Gel 10 and used this matrix to purify FK506-binding proteins from human Jurkat cell and calf thymocyte cytosols. In addition to the well characterized 12 kDa FK506 binding protein, FKBP-12, they recovered a ~60 kDa protein which they showed had the same $NH_2$-terminal sequence (15 residues) as the sequence we had previously published for hsp56[32]. Proteins that bind immunosuppressant drugs like FK506, rapamycin or cyclosporin A are called immunophilins, and all known immunophilins possess rotamase (peptdyl-prolyl *cis-trans* isomerase) activity *in vitro*. The hsp56 was also found to have homology to the immunophilins FKBP-12 and FKBP-13, supporting the notion that hsp56 may be a member of the immunophilin/rotamase protein family. Simultaneously, Lebeau *et al.*[36] used the EC1 antibody prepared by Faber to screen a rabbit liver cDNA library and clone the cDNA for hsp56. They found that the segment between amino acids 41 and 137 of the protein had 55% amino acid homology to rotamase. Thus, it seems very likely that hsp56 is a rotamase and it is of special interest that three potential protein *chaperones*, hsp90, hsp70 and hsp56, appear to exist together in a complex where their functions might complement each other.

Our immunoadsorption results with the EC1 antibody against hsp56 strongly indicated that the three heat shock proteins exist together in a cytosolic complex independently of the presence of any steroid receptor and that the complex is present in considerable stoichiometric excess of receptors[32]. Perdew and Whitelaw[37] have used coimmunoadsorption with a monoclonal antibody directed against hsp90 to demonstrate that hsp90 exists in Hepa cell cytosol in a heteromeric complex containing proteins of relative molecular weights 68,000, 63,000, 56,000 and 50,000. The 68 and 56 kDa proteins were identified as hsp70 and hsp56. The 50 kDa protein was shown to be the same as the 50 kDa protein that coimmunoadsorbs with the $pp60^{v\text{-}src}$-hsp90 complex. It has not yet been determined if this 50 kDa protein is the same as the 50 kDa protein found in association with the untransformed progesterone receptor complex[23], but it seems likely.

## Is the Docking Complex a Protein Transport Unit - a Transportosome?

The concept that has developed is that the steroid receptors

are associated with a multiprotein docking complex that performs a very general and essential function in protein folding and trafficking. A minimal protein transport unit containing the proteins discussed above is illustrated in Fig. 1 and I have called the unit a *transportosome*. The stoichiometry reflects generally the stoichiometry determind for cytosolic receptor complexes. Metabolic labeling and cross-linking studies from a number of laboratories have determined that the core complex contains 2 molecules of hsp90 per molecule of glucocorticoid or progesterone receptor (see Alexis *et al.*[29] and Rexin *et al.*[30] and multiple references there in). The hsp90 can be purified to homogeneity as a dimer, thus its presence as a dimer in such a minimal complex is predictable. Cross-linking procedures were used by Gehring's labortory to determine that there is one molecule of hsp56 per glucocorticoid receptor in the untransformed cytosolic complex[30], and that the receptor exists in whole cells as a high molecular weight structure of Stokes radius 82 Å with the same subunit structure as the cytosolic complex – 1 receptor, 2 hsp90 and 1 hsp56[38]. Although hsp56 is clearly bound to hsp90, Alexis *et al.*[29] and Rexin *et al.*[30] have also cross-linked it to the receptor, suggesting that it lies in close proximity to the receptor in the complex, as indicated in Fig. 1. Little is known about the stoichiometry of p50, p23 and p14, although Smith *et al.*[23] have been able to make crude estimates of stoichiometry for two of them in the progesterone receptor complex. It is worth noting again that, in addition to hsp56, there are smaller FK506 binding proteins of ~23 kDa and 12-13 kDa. It is intriguing to consider the possibility that these immunophilins are the p23 and p14 proteins that have been reported in steroid receptor complexes. It is not unreasonable to speculate that, in addition to a conserved rotamase activity, these immunophillins might have a conserved ability to bind hsp90, perhaps even influencing its function.

## Linkage Between Protein Folding and Transport

The transport form of the steroid receptor shown in Fig. 1 does not contain hsp70. I have not included hsp70 because the Figure is intended to reflect transport in the cytoplasm, and, to date, hsp70 has not been identified in receptor complexes prepared from hormone-free cells where the receptor is located in the cytoplasm. However, there are clear indications that the unfoldase activity of hsp70 is required for the

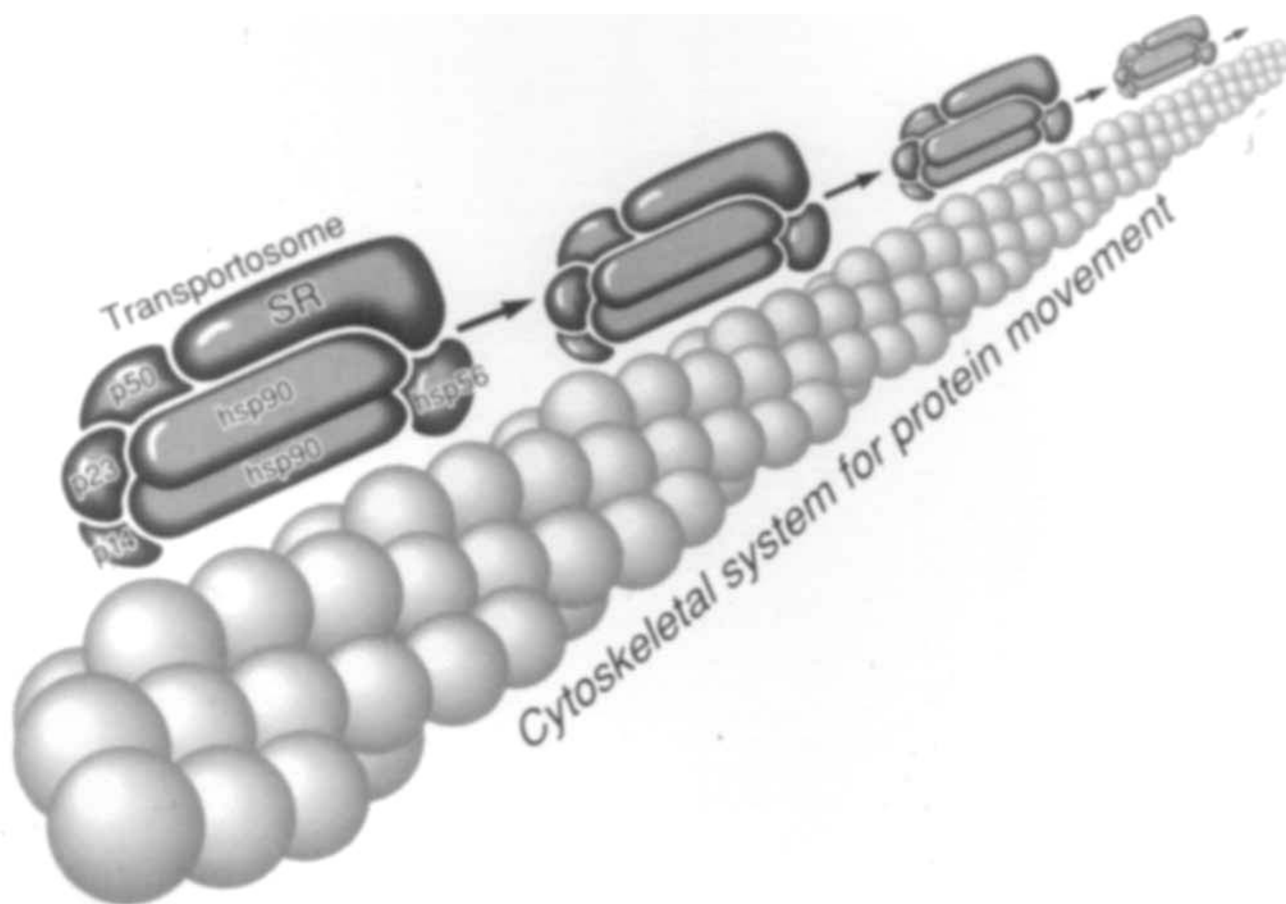


**Fig. 1.** The steroid receptor heteroprotein complex as it may function as a transportosome in the intact cell. SR = steroid receptor. The other receptor-associated proteins are discussed in the text.

initial attachment of the receptor to the transportosome. The evidence is derived from cell-free studies of receptor heterocomplex reconstitution.

Despite the fact that steroid receptors are bound tightly to hsp90, the association does not reflect a free equilibrium. It seems that, when the receptor is dissociated from hsp90, its conformation is changed (as indicated in Fig. 2) such that it cannot spontaneously reassociate with the heat shock protein in a nonenzymatic manner. In the case of the glucocorticoid receptor (but not progesterone or estrogen receptors), this conformational change eliminates steroid binding activity and it is only the hsp90-bound form that has a high-affinity steroid-binding conformation[39]. In the first cell-free demonstration of receptor association with hsp90, glucocorticoid receptors were translated in rabbit reticulocyte lysate and binding of the translation product to hsp90 was demonstrated by formation of a 9S receptor complex[40] or by coimmunoadsorption of receptor with a monoclonal anti-hsp90 antibody[41]. Importantly, it was found that the receptor binds to hsp90 at or near the termination of receptor translation[41].

Smith *et al.*[18] established that receptor association with hsp90 in rabbit reticylocyte lysate is not obligatorily coupled to receptor translation when they showed that incubation of immunopurified chick progesterone receptor with reticulocyte lysate results in binding of the avian receptor to rabbit hsp90. Subsequent studies showed that incubation of the immunopurified mouse glucocorticoid receptor or the oncogenic tyrosine kinase $pp60^{v\text{-}src}$ with reticulocyte lysate results in heterocomplex formation with hsp90[24,42]. The lysate-mediated reconstitution of hsp90 heterocomplexes is ATP-dependent and has a monvalent cation requirement similar to that shown for the clathrin unfoldase activity of hsp70[43,44]. In all cases where receptor or $pp60^{v\text{-}src}$ is reconstituted with hsp90 by reticulocyte lysate, hsp70 is also bound[42,43,44], and Smith *et al.*[43] have shown that addition of a monoclonal antibody against hsp70 to reticulocyte lysate inhibits hsp90 reassociation with the progesterone receptor. Taken together, these observations support the proposal that the protein unfoldase activity of hsp70 is required for associating the receptors or $pp60^{v\text{-}src}$ with hsp90.

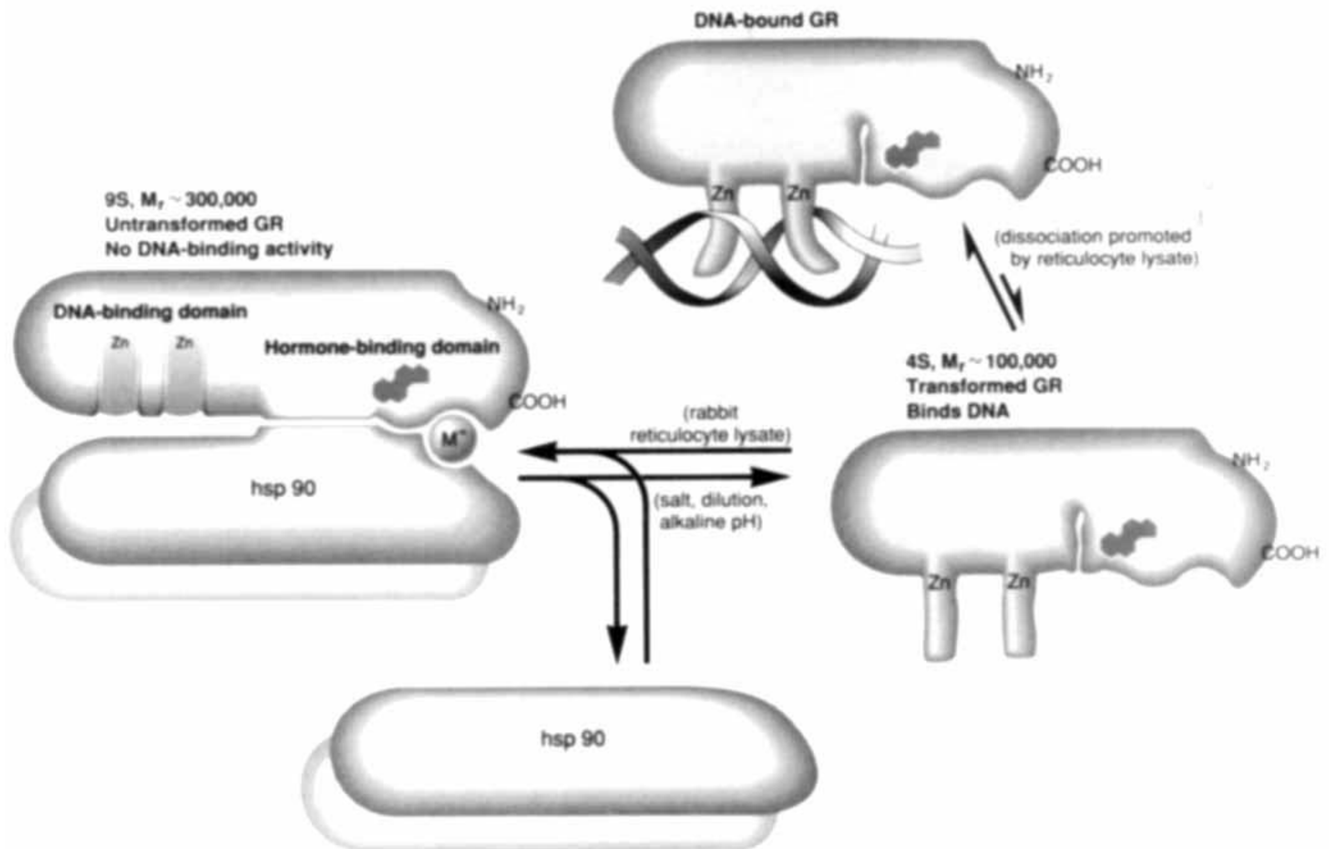


**Fig. 2.** Model of dissociation and reconstitution of the glucocorticoid receptor-hsp90 interaction. Hsp90 binds to the hormone binding domain of the receptor to form a complex without DNA binding activity which is stabilized by molybdate (indicated by the *small globe* with the *M*=). Addition of salt to cytosol, dilution or alkaline pH promotes hsp90 dissociation and simultaneous transformation to the DNA binding state (indicated by the receptor with exposed 'zinc fingers' on the right). Either immunoadsorbed or DNA-bound unliganded receptor is reassociated with hsp90 in an ATP-dependent reaction directed by rabbit reticulocyte lysate, to reconstitute the non-DNA binding, untransformed receptor. Dissociation of the untransformed receptor-hsp90 complex is physiologically induced by steroid binding. That mechanism is not indicated in the Figure because only the steroid-free receptor is reconstituted into the heterocomplex with hsp90 by reticulocyte lysate (From Scherrer *et al.*[26], Fig. 4). Reproduced by permission of the American Society for Biochemistry and Molecular Biology.

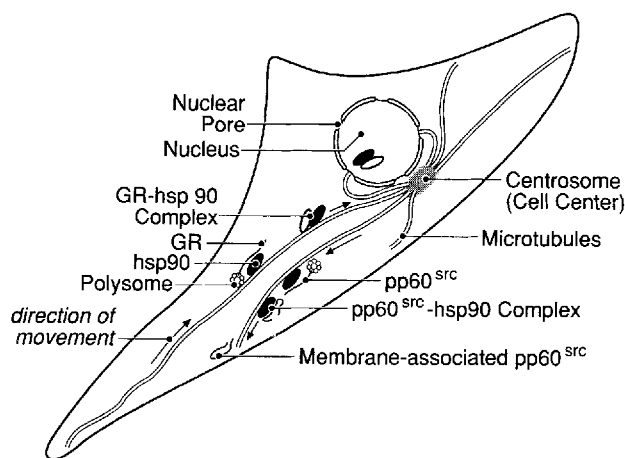


**Fig. 3.** Bidirectional movement of proteins bound to hsp90. Binding of proteins to hsp90 appears to occur very close to or at the time of their translation. The hsp90-containing structures function as transportosomes and they retain the protein during cytoplasmic transport. Systems exist for transport in both directions (i.e. toward the nucleus and away from it), with the direction of movement being determined by targeting signals inherent to the transported protein, such as the nuclear localization signals of steroid receptors. Although movement is along cytoskeletal protein scaffolds, it is not yet known whether the steroid receptors or $pp60^{src}$ move along tubulin- or actin-based scaffolds. GR = glucocorticoid receptor.

It is important to note that when a receptor or $pp60^{v\text{-}src}$ is reconstituted with hsp90 in the reticulocyte lysate system, it is bound to a multiprotein complex that contains other proteins in addition to hsp70; this is indicated by the presence of p23 in the reconstituted progesterone receptor heterocomplex[43] and the presence of p50 in the reconstituted $pp60^{v\text{-}src}$ heterocomplex[42]. In the glucocorticoid receptor system it has been shown that the reconstituted heterocomplex restores receptor function to that of the 9S form[24]. That is, reassociation of hsp90 is accompanied by conversion of the receptor back to a form that binds steroid with high affinity but does not bind to DNA.

From the above, it seems quite reasonable to suggest that protein unfoldase enzyme activity is required to associate a steroid receptor or $pp60^{v\text{-}src}$ with the hsp90-containing heterocomplex. One can then ask, when does attachment to such a complex take place? Early studies in intact cells showed that as soon as $pp60^{v\text{-}src}$ is translated, it becomes associated hsp90 and p50. The $pp60^{v\text{-}src}$ remains associated with these proteins in a cytosolic complex until it localizes to the plasma membrane. Thus it has been thought that hsp90 and p50 are involved in translocation of soluble $pp60^{v\text{-}src}$ to cell membranes (see Brugge[45] for review). It has been established that newly synthesized glucocorticoid receptors are bound to hsp90 in intact lymphocytes[46], and association with hsp90 occurs at or near the termination of receptor translation in reticulocyte lysate[41]. Thus, it is intended in Fig. 3, to indicate that both $pp60^{src}$ and the glucocorticoid receptor are bound to the hsp90 component of the transport system in a manner that is linked to their translation in the intact cell. Members of the hsp70 family have been shown to interact co-translationally with many proteins[47]. Such co-translational interaction would be consistent with the proposed role for the protein unfoldase activity of hsp70 in protein attachment to the transportosome complex.

## General Speculations

It is well established that particles move in the cytoplasm and axoplasm along cytoskeletal protein scaffolds, and there is limited evidence for the association of both hsp90 and steroid receptors with cytoskeletal structures in intact cells. For example, hsp90 has been localized by immunofluorescence to filamentous cytoplasmic structures distributing in microtubular fashion in several types of cells[48,49]. Also, immunofluorescence studies utilizing a monospecific monoclonal antibody against the glucocorticoid receptor localized the receptor to cytoplasmic fibrillar structures in both human uterine carcinoma cells[50] and human gingival fibroblasts[51]. As with hsp90, the distribution of the receptor immunofluorescence was the same as tubulin immunofluorescence as determined in the same cells by a double immunofluorescence procedure.

In cytoplasm and axoplasm, particle movement occurs both towards the nucleus and away from it, as indicated in Fig. 3. In the model proposed here, targeted proteins would move along these pathways while attached to transportosomes. The proposed transportosome composition (shown in Fig. 1) is derived almost entirely from studies of steroid receptor-associated proteins. Steroid receptors are known to move in both directions, i.e. into the nucleus and from the nucleus back into the cytoplasm. This receptor cycle was originally conceived by Allan Munck (see Orti *et al.*[52] for review), and the shuttling of receptors into and out of the nucleus in intact cells has recently been elegantly demonstrated by the laboratories of Edwin Milgrom[53] and Don DeFranco[54].

With the transportosome model, one can easily conceive of a class of transcription factors that shuttle information from the plasma membrane to the nucleus. For example, a protein that regulates transcription, could be bound co-translationally to hsp90 in the transportosome complex, and as happens with the glucocorticoid receptor, its nuclear localization signal could be repressed by binding to hsp90. A second targeting signal or some general characteristic of the molecule might direct its passage to the end of the outward movement pathway. Thus, it would move to the inner surface of the plasma membrane where it remains *docked* to the transportosome. Some event, such as phosphorylation by a hormone-bound plasma membrane receptor, could then favor dissociation from hsp90. Now, much as happens with the glucocorticoid receptor after steroid binding, the nuclear localization signal is derepressed, permitting the protein to shuttle rapidly to the nucleus where it activates transcription. This kind of hsp90-regulated shuttling mechanism could fill in a large gap in our understanding of how the interaction of peptide hormones and growth factors with plasma membrane receptors can directly regulate gene transcription.

Given the abundance, ubiquity and conservation of hsp90, it seems quite possible that a large number of proteins could

bind co-translationally to hsp90 via relatively weak forces, but forces sufficient to permit binding to the transportosome during transport in the cell. At the termini of the transport pathways, proteins may readily dissociate and be handed on to their sites of action. Such transient association with hsp90 may occur with unliganded thyroid hormone and retinoic acid receptors, for example, as they are transported to their nuclear sites of action. It is reasonable to propose that the steroid receptors have exploited such a general role of hsp90 as a protein chaparone component of the transport machinery. Thus, in binding with high affinity to hsp90 and remaining docked to the transport complex, the steroid receptors have evolved a method of signal transduction in which their dissociation from this primitive and necessary component of the system is under hormonal control.

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