MINIREVIEW

The hsp90-based Chaperone System: Involvement in Signal Transduction from a Variety of Hormone and Growth Factor Receptors (44252)

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Abstract. **A variety of transcription factors and protein kinases involved in signal transduction are recovered from cells in heterocomplexes containing the abundant protein chaperone hsp90. Genetic studies in yeast have demonstrated that binding of steroid receptors, the dioxin receptor, and some protein kinases to hsp90 is critical for their signal transducing function *in vivo.* These heterocomplexes are formed by a multiprotein chaperone machinery consisting of at least four ubiquitous proteins—hsp90, hsp70, p60 and p23. Four high-molecular-weight immunophilins have been discovered as components of steroid receptor or other transcription factor complexes with hsp90. The immunophilins, protein chaperones with prolyl isomerase activity, bind the immunosuppressant drugs FK506 or CyP-40. These immunophilins all bind *via* tetratricopeptide repeat (TPR) domains to a single TPR binding site on each hsp90 dimer, and multiple heterocomplexes exist for each protein chaperoned by hsp90 according to the immunophilin that is bound to this TPR binding site at any time. Three components of the MAP kinase signalling system (Src, Raf, and Mek) exist in complexes with hsp90 and a 50-kDa protein that is the mammalian homolog of the yeast cell cycle control protein cdc37. The $p50^{cdc37}$ binds to hsp90 at a site that is close to but different from the TPR binding site of the immunophilins, and like the immunophilins, $p50^{cdc37}$ is thought to be involved in targeting and trafficking of the protein kinases. The recent introduction of the benzoquinone antibiotic geldanamycin has facilitated the identification of proteins that are chaperoned by the hsp90-based system. Geldanamycin binds to members of the hsp90 protein family, blocking assembly of hsp90 heterocomplexes and destabilizing preformed heterocomplexes. In the presence of geldanamycin, the function of hsp90-chaperoned proteins is disrupted, and the proteins undergo rapid degradation by an ubiquitin-dependent proteasomal mechanism. It is becoming clear that hsp90 chaperoning is not only essential to a variety of signal transduction pathways, but is critical for proper folding, stabilization, and trafficking of an expanding list of proteins.** [P.S.E.B.M. 1998, Vol 217]

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0037-9727/98/2174–0420$10.50/0

Hsp90 is a highly conserved and ubiquitously expressed stress protein of animal and plant cells (1). Despite its abundance (accounting for 1%–2% of the cytosolic protein in unstressed mammalian cells) and its ability to function *in vitro* as a molecular chaperone that facilitates the folding of a variety of proteins (2), the func-

tion of hsp90 *in vivo* is still not defined. Like some other chaperones, hsp90 has the ability to interact with a wide variety of proteins regardless of their structure, affecting their activity, turnover, and cellular localization. Of particular interest are a series of recent reports implicating hsp90 and its associated proteins in signal transduction from a variety of receptors.

Since 1981, when the oncogenic tyrosine kinase $pp60^{v\text{-}src}$ (Src) was shown to be associated with hsp90 (3), 14 transcription factors and 13 protein kinases have been found to exist in native complexes with hsp90 (Table 1). The most studied of the hsp90-associated proteins are the steroid receptors, and it was the study of these complexes that led to the discovery of the hsp90-based protein chaperone system and to the discovery of the high-molecular-weight immunophilins. The high-molecular-weight immunophilins are hsp90-bound chaperones with peptidylprolyl isomerase (PPIase) activity that are pharmacologically-specific receptors for the potent immunosuppressive drugs FK506 and cyclosporin A. The interaction of steroid receptors with heat shock protein and immunophilin chaperones is the subject of two recent comprehensive reviews (4, 5) as well as several short reviews (6–8).

For most of the proteins listed in Table I, protein·hsp90 heterocomplexes were identified by copurification. Recently, it has been shown that the benzoquinone ansamycin antibiotic geldanamycin binds to hsp90 (44) and disrupts signalling *via* steroid (45) and epidermal growth factor (42) receptors. Geldanamycin has been used to demonstrate hsp90 association with mutants of the p53 tumor suppressor (25) and the reverse transcriptase of hepatitis B virus (23). Since administration of geldanamycin to intact cells is followed by rapid and selective degradation of hsp90-bound proteins, the use of this antibiotic should accelerate the identification of additional proteins that are chaperoned by hsp90.

Assembly of Heterocomplexes with Hsp90

During the 1980s, it was established that the 9S, untransformed (i.e., non-DNA-binding) form of steroid receptors is a heterocomplex containing one molecule of receptor, two molecules of hsp90 and one molecule of an immunophilin (4). It rapidly became clear that steroid receptors did not bind hsp90 in a simple equilibrium interaction, and after the failure of multiple attempts to form receptor·hsp90 complexes under cell-free conditions, it was found that glucocorticoid receptors (GR) translated in rabbit reticulocyte lysate formed GR·hsp90 heterocomplexes that were both physically and functionally indistinguishable from the native 9S form of the receptor (46, 47). Smith *et al.* (48) then showed that avian progesterone receptors (PR) that were immunoadsorbed from cytosol and stripped of their associated proteins by washing with high salt buffer would form a heterocomplex with rabbit hsp90 when they were incubated with rabbit reticulocyte lysate. Scherrer *et al.* (49) showed that reconstitution of GR·hsp90 complexes with reticulocyte lysate was accompanied by conversion of the receptor from the DNA-binding form back to the non-DNA-binding form.

Although most of the published experiments on cell-free receptor·hsp90 heterocomplex assembly have used commercial preparations of rabbit reticulocyte lysate that are commonly used for protein translation, the hsp90 heterocomplex assembly system appears to be ubiquitous in that concentrated lysates from a variety of mammalian, insect, and plant cells can form functionally normal GR·hsp90 heterocomplexes (50). In addition to forming complexes between hsp90 and all of the steroid receptors (4), the re-

Table I. Proteins that are Chaperoned by hsp90

Substrate protein	Reference	Substrate protein	Reference
Transcription factors		**Protein kinases**	
Glucocorticoid receptor	9	Tyrosine kinases	
Progesterone receptor	10,11	v-Src, c-Src	26,27,28
Estrogen receptor	12,13	v-fps	29
Androgen receptor	12,14	v-yes	29
Mineralocorticoid receptor	15	v-fes	29
v-erbA	16	v-frg, c-frg	30,31
Dioxin receptor	17,18	lck	31
Sim	19	Wee1 kinase	32
MyoD1[a]	20	Sevenless PTK[b]	33
E12[a]	20	Heme-regulated eIF-2α kinase	34,35
Heat shock factor	21	eEF-2 kinase	36
Tumor promotor-specific binding protein	22	Casein kinase II	37,38
Hepatitis B virus reverse transcriptase	23	v-Raf, c-Raf, Gag-Mil	39,40,41
p53 tumor suppressor mutant	24,25	Mek	42
		PI-4 kinase[c]	43

Note. The list includes only proteins that bind to the chaperone or "substrate" site on hsp90. The Table is a portion of Table I from Pratt and Toft (4).

[a] Only functional interaction has been demonstrated.

[b] Interaction suggested on the basis that hsp90 mutation impairs function.

[c] hsp90 copurifies with the protein.

ticulocyte lysate system has been used to assemble hsp90 complexes with the dioxin receptor (51) and the protein kinases Src (28), Raf (39), and Mek (42). In general, the assembly of complexes has been assayed directly by demonstrating the binding of hsp90 to an immobilized protein by immunoblotting. Because the hormone binding domain (HBD) of the GR (52) and some of the other steroid receptors (4) must be bound to hsp90 to be in a conformation that binds steroid initially, incubation of the receptors with reticulocyte lysate converts these receptors from a nonsteroid binding state to a steroid binding state (49). Thus, the generation of steroid binding activity can be used as a rapid protein 'folding' assay to detect the formation of GR·hsp90 complexes in which the HBD is in the high affinity steroid binding conformation. Using both formation of steroid binding sites and direct measurement of the formation of receptor·hsp90 complexes, a number of details of the heterocomplex assembly process have been worked out and are summarized in Figure 1 (53).

Assembly requires ATP/Mg^{2+} and is monovalent cation-dependent, with K^+, NH_4^+, and Rb^+ permitting assembly and Na^+ and Li^+ being inactive (54, 55). The action of hsp70 has a similar monovalent cation selectivity (56). Members of the hsp70 family chaperone protein folding (57), and hsp70 is required for receptor·hsp90 heterocomplex assembly by reticulocyte lysate (54, 58). Some hsp70 is always present in the final GR·hsp90 heterocomplex assembled by reticulocyte lysate, but depending upon the cell type, it may or may not be present in native receptor heterocomplexes isolated from cytosols (4).

As indicated in Figure 1, the first step in the assembly process appears to be the formation of a multiprotein chaperoning complex that we have called a *foldosome* (59). Although hsp70 is found in cytosolic complexes with hsp90 independent of the presence of steroid receptors (60, 61), purified hsp70 and hsp90 do not bind to each other unless a third factor in cell lysates is present (62). Smith *et al.* (63) predicted that the combining factor was a 60-kDa protein (p60) that coimmunoadsorbed with hsp90 and hsp70 and was found in PR·hsp90 heterocomplexes assembled in reticulocyte lysate when ATP was limiting (54) or at early times of assembly (64). Human p60 was cloned by Honoré *et al.* (65) and shown to be the homolog of the nonessential yeast stress protein Sti1 (66). Chen *et al.* (67) have shown that p60 binds independently to hsp70 *via* an N-terminal TPR (tetratricopeptide repeat) region and to hsp90 *via* a central TPR region to form an hsp90·p60·hsp70 complex. Like hsp70, p60 is required for GR·hsp90 heterocomplex assembly (68). We have recently shown that mixing purified rabbit hsp90 and hsp70 with bacterially expressed p60 produces an hsp90·p60·hsp70 complex that converts the GR from a nonsteroid binding to a steroid binding form (Step 2 in Figure 1) (53).

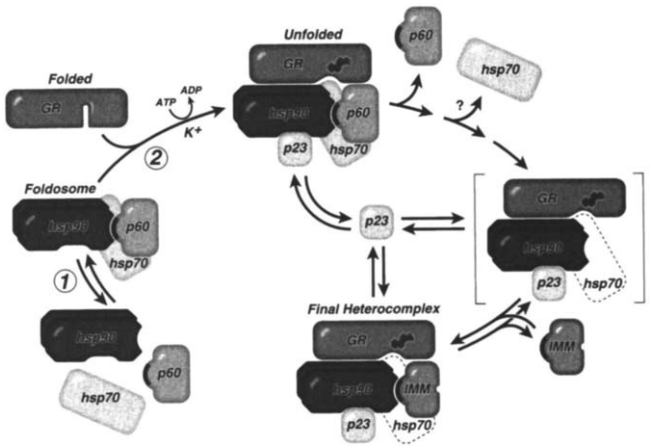

Figure 1. Model of GR·hsp90 heterocomplex assembly. In the model the hsp90-based chaperone system is viewed as converting the GR from a folded conformation in which the hydrophobic steroid binding pocket is not accessible to hormone to a partially unfolded conformation with an accessible steroid binding site (indicated by the steroid structure). IMM stands for an immunophilin such as FKBP52 or CyP-40. The immunophilins and p60 bind to hsp90 *via* TPR domains, which are indicated by the solid black crescents on these proteins. The dashed line for hsp70 indicates its optional presence in native GR·hsp90 heterocomplexes. Other details are summarized in the text. (From Dittmar *et al.* (191) with permission).

In Figure 1, the GR that is free of accessory proteins is considered to have its hormone binding domain in a 'folded' state in which the hydrophobic steroid binding pocket is buried and not accessible to steroid. In the hsp90-bound state, the HBD is envisioned as being partially 'unfolded' such that the hydrophobic steroid binding pocket is opened up and accessible to steroid. It has been shown that thiol moieties in the GR HBD that are accessible to a chemical derivatizing agent when the receptor is bound to hsp90 are less accessible, or not accessible, after hsp90 dissociation (69). This observation is consistent with a model in which the hsp90-free HBD is in a more folded state than the hsp90-bound HBD. If this aspect of the model is correct, it means that the complex of hsp90·p60·hsp70 may somehow promote a partial unfolding of the HBD. Such a role for the hsp90·p60·hsp70 complex in assisting unfolding would be very different from a pure chaperone function in which the chaperones facilitate proper folding of a previously unfolded protein.

Although a mixture of purified rabbit hsp90 and hsp70 and bacterially expressed human p60 can alter the folding state of the GR HBD such that it binds steroid with high affinity, the steroid binding sites that are formed are highly unstable unless a fourth protein called p23 is present (53). This component of the chaperone system is a widely distributed, acidic 23-kDa protein (70) that binds directly to hsp90 in an ATP-dependent manner (71). Depletion of p23 from reticulocyte lysate (72) or from immunoadsorbed hsp90 multiprotein chaperoning complexes (73) prevents stable receptor·hsp90 heterocomplex assembly, and readdition of purified human p23 restores assembly activity. At this time, it is unclear how the binding of p23 to hsp90 stabilizes the GR·hsp90 complex or at least a functional (i.e., steroid-binding) state of the complex, but some transition metal oxyanions such as molybdate can interact with

hsp90 and do the same thing (53), perhaps by acting as an analog of phosphate.

The four-protein system of hsp90, p60, hsp70, and p23 yields a heterocomplex that still contains p60 (53); to achieve the final heterocomplex, p60 must dissociate. The mechanism of p60 exit from the heterocomplex is unknown, but it appears that reticulocyte lysate has a factor(s) that facilitates p60 release (53). The system shown in Figure 1 is only a minimal heterocomplex reconstitution system. For example, it does not contain a 48-kDa protein that is recovered in PR heterocomplexes at early times of assembly in reticulocyte lysate (64). The cDNA for this 48-kDa protein was recently cloned (74) and shown to be the hsc70-interacting protein, Hip, reported by Höhfeld *et al.* (75). To date, no role for Hip in heterocomplex assembly has been defined, but it is likely to be a component of the complete heterocomplex assembly system. Because mutations in the yeast DnaJ homolog Ydj1 can affect steroid receptor function (76, 77), it is reasonable to predict that hsp40, a family of proteins that are mammalian DnaJ homologs, is critical for optimal GR·hsp90 heterocomplex assembly in intact cells. Some hsp40 is present in our purified preparations of hsp70, yielding low levels in our reconstituted minimal heterocomplex assembly system, and it could be that hsp40 is required for step 2 in the scheme of Figure 1.

The hsp90-based heterocomplex assembly system appears to be a very basic cellular process. Not only is the system apparently ubiquitous in eukaryotes (50), but there is functional conservation of activity between the plant and animal kingdoms. For example, human p23 binds to plant hsp90 in an ATP-dependent manner (78) and stabilizes GR·plant hsp90 complexes formed in wheat germ extract (73). Also, purified wheat hsp70 is as active as mouse hsp70 in supporting GR·hsp90 heterocomplex assembly by hsp70-depleted rabbit reticulocyte lysate (50). Recently, we have found that a purified recombinant plant hsp90 is fully active at reconstituting the steroid-binding state of the GR when combined with purified rabbit hsp70 and human p60 and p23 (191). This conservation of function between the plant and animal proteins that are required for heterocomplex assembly suggests that assembly of protein heterocomplexes with hsp90 is fundamental to the biology of all eukaryotic cells.

Hsp90 and Steroid Receptor Function

The steroid receptors are direct signal transducers in that they have both a hormone-binding site that receives the signal, and in their hormone-activated state they transduce the signal by binding to response elements in the genome where they alter the transcription rates of specific genes. The receptors are divided into three separate domains that determine hormone binding, DNA binding and transcriptional activating activity (79). In addition to determining hormone binding, the HBD contains a dimerization site and the AF2 autonomous transactivation domain. Hsp90 is bound to the HBD, and at least for glucocorticoid, mineralocorticoid and progesterone receptors, hsp90 heterocomplex assembly is required to achieve the proper folding state for high-affinity steroid binding (4). Because deletion of the HBD yielded receptors that were constitutive activators of transcription, it was realized that the HBD regulates the transcriptional activating activity of the rest of the receptor (79). There is considerable evidence that the ability of the HBD to regulate the activity of the rest of the receptor is related to its binding to hsp90 (4).

Some of the most important experiments showing a regulatory role for hsp90 were performed with fusion proteins containing a steroid receptor HBD (80). Picard *et al.* (81) first demonstrated that the regulatory function of the HBD is transferable when they showed that fusion of a steroid receptor HBD with a nonreceptor protein conferred hormone responsiveness onto the function of the fusion protein. Yamamoto and his colleagues proposed that repression of fusion protein function is conferred *via* association of the receptor HBD component with hsp90 (8, 81), and it has been shown that such HBD fusion proteins are recovered from the cytosol of transfected cells in native heterocomplexes with hsp90 (82). Importantly, studies involving the genetic manipulation of chaperone activity in yeast demonstrate that hsp90 plays a critical role in determining hormone responsiveness of steroid receptors (76, 77, 83, 84). Interestingly, the HBD does not have to be covalently linked to the rest of the receptor to confer hormone responsiveness. Spanjaard and Chin (85) have reconstituted hormone-dependent GR activity by expressing as independent proteins a fragment of the GR containing only the HBD and a fragment containing the rest of the GR without the HBD, with each fragment being fused to either a c-Jun or c-Fos leucine zipper.

Although it is clear that steroid receptor·hsp90 heterocomplex assembly is required for hormonal regulation of receptor activity, it is not clear how that regulation is brought about. The model that is now prevalent in reviews and in the textbooks is that steroid receptors in hormone-free cells are located either in the cytoplasm (e.g., glucocorticoid and mineralocorticoid receptors) or in the nucleus (e.g., estrogen and progesterone receptors) in heterocomplexes with hsp90 (i.e., the final heterocomplex of Figure 1). Binding of hormone to the receptors promotes their dissociation from hsp90, which is followed by receptor dimerization and association with appropriate response elements in the chromatin (4). Thus, the generally accepted model assumes that the cytoplasmic GR, for example, moves to the nucleus only after it has undergone steroid-dependent dissociation from hsp90. This model of steroid hormone action is based on the observation that receptors that had undergone steroid-dependent transformation *in vivo* or *in vitro* were no longer recovered as large (~9S) complexes that contain hsp90 (4).

This traditional model is currently in the process of some revision. A glaring gap that this model shares with the models of signal transduction from membrane receptors to

nuclear effectors is that there is no explanation of how the signal trafficks through the cytoplasm to the nucleus. In the case of the transformed GR, the signal trafficks in the form of the receptor itself, and in the case of signalling from membrane receptors, it is the activated signalling protein, such as phosphorylated Erk (MAP kinase) or STAT protein complexes, that must traffick to the nucleus. In neither case is it known how the trafficking occurs. One notion regarding trafficking of the steroid-transformed GR is that association of the receptor with hsp90 is required for its cytoplasmic-nuclear movement (86). This idea is supported by experiments in intact cells that show that molybdate (87), which stabilizes receptor·hsp90 complexes, and geldanamycin (88), which blocks receptor heterocomplex assembly, both inhibit steroid-dependent GR trafficking to the nucleus. These observations support a model in which dynamic association of steroid-bound receptors with hsp90 is required for their trafficking. Yang and DeFranco (87) have suggested that dynamic association with hsp90 is required to permit the access of macromolecules that in essence link the receptor to a movement machinery, and we have suggested that the hsp90-bound immunophilins may perform that linkage function (89).

It seems clear that binding to hsp90 affects the GR in several ways. Passage of the hormone-free receptor through the heterocomplex assembly cycle shown in Figure 1 yields a tight GR·hsp90 heterocomplex in which the HBD is in a high-affinity steroid-binding state. When it is in this tight complex, the two nuclear localization signals (NLS) of the GR are blocked, or are in some way nonfunctional, and the great majority of the receptor is cytoplasmic. This is not true for progesterone or estrogen receptors where the NLS is functional when the receptor is bound to hsp90, and the hormone-free receptors localize to the nucleus in the heterocomplex form. When held in tight association with hsp90, the steroid receptors and chimaeras containing an otherwise constitutively active transcription factor fused to a receptor HBD are unable to get to their final destinations within the nuclear matrix. However, when steroid binds to the HBD, there is a conformational change in the HBD that transforms the very tight interaction with hsp90 to a weak interaction that may be typical of many proteins chaperoned by the hsp90 system. Because the transformed, steroid-bound receptor interacts only weakly with hsp90, the receptor may be readily "handed off" to nuclear acceptors at the termini of the trafficking system (89).

Although the details of the heterocomplex assembly process shown in Figure 1 are being worked out, we are only beginning to conceive of why the steroid receptors form tight heterocomplexes with hsp90. In addition to performing critical roles in hormone binding, hormonal regulation and probably trafficking of steroid receptors, binding to hsp90 stabilizes the receptors to proteolytic degradation (45, 88). Indeed, one gets the impression that for the steroid receptors, the hsp90-based chaperone machinery may function as a 'cradle-to-grave' (i.e., translation to degradation) protein management system.

The Immunophilins

The immunophilins, protein chaperones with peptidylprolyl isomerase (PPIase) activity (90, 91), are widely distributed in both plant and animal cells. There are two classes of immunophilins—the FKBPs, which bind immunosuppressant drugs of the FK506 type, and the cyclophilins (CyPs), which bind cyclosporin A. The immunosuppressant drugs bind to the PPIase sites on the immunophilins and inhibit *cis-trans* isomerization of peptidylprolyl bonds *in vitro*. The most studied immunophilins are the low-molecular-weight immunophilins, such as FKBP12 and CyP-18, which bind as the immunophilin-immunosuppressant drug complex to calcineurin and inhibit its phosphatase activity, an effect that is thought to block the pathway for T-cell activation (92, 93). Three high-molecular-weight immunophilins, FKBP51, FKBP52 and CyP-40, were discovered as components of steroid receptor·hsp90 heterocomplexes.

The high-molecular-weight immunophilins differ from the low-molecular-weight immunophilins in that they contain three or more tetratricopeptide repeats (TPRs) in addition to the PPIase domain. The TPR domains (indicated by the solid black crescents in Figure 1) are degenerate sequences of 34 amino acids arranged in tandem repeats that are thought to be sites where intra- and intermolecular interactions occur (94). There is a site on hsp90 that binds a variety of proteins containing TPR domains (89), and the immunophilins bind to this site (89, 95–97). Because p60 also binds to hsp90 *via* TPRs (67) and blocks the binding of immunophilins (89), immunophilins are shown entering the GR·hsp90 heterocomplex in Figure 1 only after the dissociation of p60. The final steroid receptor heterocomplex contains one molecule of receptor in association with a dimer of hsp90 and one molecule of an immunophilin (98, 99) as well as an undetermined amount of p23. This complex may (e.g., PR) or may not (e.g., GR) contain substoichiometric amounts of hsp70, depending both on the receptor and on the cell type from which it is isolated. Several high-molecular-weight immunophilins have been identified in steroid receptor heterocomplexes, but as two immunophilins cannot exist in one complex at the same time, there are multiple heterocomplexes for each receptor (100, 101). Because the TPR domain proteins bind in a readily reversible manner, a single receptor·hsp90 unit can be associated with several different TPR domain proteins over time. Thus, the final heterocomplex should be regarded as a highly dynamic entity.

FKBPs. In 1992, a 59-kDa protein that was known to be both a common component of untransformed steroid-receptor complexes (102) and a stress protein (103) was shown to bind to FK506 and rapamycin affinity matrices (104, 105). cDNAs encoding the protein were cloned (106–108), and because the human protein has a predicted mo-

lecular weight of 51,810 (107), the term FKBP52 has replaced former terminology (e.g., p59, p56, hsp56, HBI). FKBP52 was known to exist in heterocomplexes with hsp90 independent of steroid receptors (60–62), and Radanyi *et al.* (95) were the first to demonstrate that the FKBP52 TPR domain was required for its binding to hsp90. FKBP52 has been found in all species tested, and it may be ubiquitous. Binding of FKBPs to hsp90 *via* TPR domains is a conserved protein interaction in plants, suggesting that the formation of these complexes is fundamental to the biology of eukaryotic cells (78).

A 54-kDa protein reported by Smith *et al.* (109) to copurify with the chicken progesterone receptor was shown to possess high amino acid homology with rabbit FKBP52 (110) and to bind to an FK506 affinity resin (111). This protein is now called FKBP51. A cDNA for human FKBP51 has been cloned, and the deduced amino acid sequence is 55% identical with that of hFKBP52 (112). FKBP51 has been identified in human as well as in avian PR heterocomplexes (113), and it appears to be expressed in all tissues (112).

The high molecular weight FKBPs possess three globular domains followed by a calmodulin binding domain at the C-terminus (114). The N-terminal domain possesses 49% homology with FKBP12 (114), has PPIase activity *in vitro* (115), and binds FK506 (116). Domain II possesses a nucleotide binding site, and domain III contains three tetratriocopeptide repeats that determine binding to hsp90. Most of the FKBP52 localizes by indirect immunofluorescence to the nucleus, with the rest being localized to microtubules in the cytoplasm (117). FKBP52 was shown by confocal imaging in the WCL2 line of Chinese hamster ovary cell to colocalize with the GR in the same mottled, nonrandom pattern throughout all planes of the nucleus but to be excluded from nucleoli (117).

CyP-40. When the cDNA encoding a 40-kDa protein that was first identified by Ratajczak *et al.* (118) as a component of bovine estrogen receptor (ER) heterocomplexes was cloned (119), it was found to be the same as a bovine 40-kDa cyclosporin A-binding protein called CyP-40 (120). The N-terminal half of CyP-40 has a high homology with CyP-18 and contains the PPIase site (119). The C-terminal half possesses 30% amino acid identity with domain III of FKBP52, and it contains three TPRs and a calmodulin binding domain (119, 121). As with FKBP52, the TPR domain is required for binding to hsp90 (89, 96, 97), and FKBP52 and CyP-40 compete with each other in binding to a common site on hsp90 (97, 101).

Protein Phosphatase 5. Three laboratories have isolated cDNAs encoding a protein serine/threonine phosphatase designated PP5 (122–124). PP5 possesses a C-terminal catalytic domain with protein phosphatase activity and an N-terminal domain with four TPRs that determine its binding to hsp90 (125). A 55-residue connecting region between the N-terminal TPR domain and the C-terminal phosphatase domain possesses 50% amino acid homology and 22% identity with the central portion of the PPIase domain of human FKBP52 (126). Of the nine residues in this region of FKBP52 that are involved in high-affinity interactions with FK506, three residues are retained and four have homologous substitutions in PP5, and PP5 is retained by an FK506 affinity matrix (126). Thus, PP5 has the properties of a low-affinity FK506 binding immunophilin. PP5 is recovered with GR·hsp90 heterocomplexes (125), and it binds to the common immunophilin binding site on hsp90 (126). In contrast to FKBP52, PP5 is localized predominantly in the cytoplasm (M. Chinkers, personal communication).

Immunophilin Function. The function(s) of the immunophilin components of steroid receptor heterocomplexes is unknown, but several points are worth noting. Their PPIase activity suggests that the immunophilins may play a role in protein folding in the cell (91), but the GR can be properly folded and assembled into a complex with hsp90 by a purified system that does not contain any immunophilins (53, 68), and inhibition of PPIase by FK506 or cyclosporin A does not affect receptor folding by reticulocyte lysate (101). It also seems quite clear that, in contrast to FKBP12 and CyP-18, inhibition of calcineurin phosphatase is not required for the function of the high-molecular-weight immunophilins that bind to hsp90 (127, 128). Both FK506 and cyclosporin A have been reported to potentiate transcription induced by low concentrations of steroid (100, 113, 129, 130), but this effect is unrelated to the receptor-associated immunophilins. Rather, the immunosuppressants compete for the outward transport of steroid by an ATP-binding cassette transporter, and thus they increase the intracellular concentration of steroid (131, 132).

There is no clear evidence that immunophilins are required for steroid response. Warth *et al.* (133) found that a CyP-40 homolog in yeast was associated with hsp90 but was not required for steroid receptor function, whereas Duina *et al.* (134) found that null mutations of Cpr7, a more distantly related yeast CyP-40 homolog, adversely affected GR function. None of the immunophilins examined to date appear to be essential for the viability of haploid yeast cells (135), and this lack of essentiality could reflect a redundancy in immunophilin action. Transfection of the TPR domain of PP5 has been shown to inhibit GR-mediated transactivation (125), but as this domain also blocks the binding of p60 to hsp90 (126), it may be GR·hsp90 heterocomplex assembly and not immunophilin action that is the critical event inhibited.

It is possible that different arrays of immunophilins act on different receptors. In the only stoichiometry experiments performed to date, it was found that FKBPs account for 52% and PP5 for 35% of GR heterocomplexes in L cell cytosol, with CyP-40 accounting for fewer than 12% (126). On the basis of its accounting for only a minority of GR heterocomplexes and the fact that it is dispensible for GR function in yeast, it has been suggested that CyP-40 may not be relevant to GR function (89, 133). Several observations

suggest that there exist differential interactions between hsp90-associated immunophilins and target proteins such as steroid receptors. For example, Nair *et al.* (112) have reported that FKPB51 and FKBP52 bind equally well to hsp90 in a purified system whereas FKBP51 accumulates preferentially in PR·hsp90 heterocomplexes assembled in reticulocyte lysate. Also, FKBP52 associates more tightly with the GR·hsp90 complex than CyP-40 (101).

One approach to determining a physiological function for the receptor-associated immunophilins is to find proteins other than hsp90 with which they interact. Chambraud *et al.* (136) have used the N-terminal PPIase domain of FKBP52 as bait in the yeast two-hybrid system to identify a 48-kDa FKBP-associated protein, FAP48. The cDNA encoding FAP48 was cloned and shown to be unique. FAP48 binds to the PPIase site of FKBP12 as well as to FKBP52, and its binding to both immunophilins is prevented by both FK506 and rapamycin but not by cyclosporin A. FAP48 could represent an endogenous ligand that regulates FKBP cellular functions.

Immunoadsorption of Chinese hamster ovary cell cytosol with the UPJ56 antiserum to FKBP52 was accompanied by coimmunoadsorption of the dynein motor protein complex (117), an association that may be related to the microtubular localization of the minority of FKBP52 that is localized in the cytoplasm of these and other cells (117, 137, 138). One notion is that the receptor-associated immunophilins may perform a targeting function to link receptors to a protein movement system involved in cytoplasmic transport of receptor to the nucleus (139). To date, no direct binding of immunophilins to receptor proteins has been demonstrated, although Rexin *et al.* (140, 141) have cross-linked the GR heterocomplex and isolated small amounts of a 150-kDa species containing the receptor and an ~50-kDa protein that may be one or more of the high-molecular-weight immunophilins. This would suggest at least a proximity of the receptor and the immunophilin in the heterocomplex (99). The nuclear localization signal was suggested (139) as a potential site of immunophilin interaction with the receptor when it was noted that FKBP52 contains a conserved sequence of negatively charged amino acids (e.g., EDLTDDED in rabbit FKBP52 (106)) between domains I and II that is electrostatically complimentary to the receptor nuclear localization signals (e.g., the NL1 sequence RKTKKKIK of rat GR (142)). Renoir *et al.* (143) raised an antibody against the negatively charged sequence, and Czar *et al.* (144) showed that injection of the antibody into L cells impeded shift of the GR from cytoplasm to nucleus. Taken together, these observations would be consistent with a role for FKBP52 in targeting receptor movement.

Hsp90 Heterocomplexes with Other Transcription Factors

As summarized in Table I, several transcription factors that are not steroid receptors are chaperoned by hsp90. The most studied of these transcription factors is the dioxin receptor. Several other factors will be mentioned briefly as they provide examples of different ways in which hsp90 chaperoning may affect a protein's activity.

Dioxin Receptor. The dioxin receptor (DR) (also called the *Ah* receptor) mediates the induction of aryl hydrocarbon hydroxylase, a cytochrome P450 that metabolizes xenobiotics (145). The DR differs from steroid receptors in that the DNA binding domain is a basic helix-loop-helix (bHLH) rather than a double 'zinc finger' structure, and when it arrives in the nucleus, the DR forms a heterodimer with the Arnt (Aryl Hydrocarbon Receptor Nuclear Translocator) protein (146) rather than forming a homodimer.

The DR behaves like the GR in that the unliganded receptor is localized in the cytoplasm (147) in a 9S heterocomplex with hsp90 (17, 18). Also, like the GR, the DR HBD must be bound to hsp90 to be in a ligand binding conformation, and the HBD·hsp90 complex is formed by the heterocomplex assembly system of reticulocyte lysate (51, 148). The hormone binding domain of the DR is called the PAS (Per-Arnt-Sim) domain because of its homology with a domain of Arnt and the *Drosophila* factors Per and Sim (149). As with steroid receptors, the transcriptional activating activity of the DR is regulated by hsp90 in a ligand-dependent manner, and the PAS domain of the DR can confer ligand dependent response onto a fusion protein (150). It has also been shown that hsp90 is essential for DR signaling in a yeast expression system (151).

Although access to the 'zinc finger' DNA binding domain is blocked when hsp90 is bound to the steroid receptor HBD, no direct interaction between hsp90 and the DNA binding domain has been detected. In contrast, the bHLH DNA binding domain of the DR forms a complex with hsp90 that is stable enough to be detected by coimmunoadsorption (152). The bHLH domain confers hsp90 binding onto a DR bHLH-Arnt chimera translated in reticulocyte lysate, and Antonsson *et al.* (153) have suggested a role for hsp90 in chaperoning a DNA-binding conformation of the DR bHLH domain.

Several other members of the bHLH-PAS family form stable complexes with hsp90; these include Sim (19) and MOP1, 3 and 4 (154). As there are numerous orphan nuclear receptors that have been cloned (155), many more hsp90-associated transcription factors will probably be identified in the future. It should be noted that hsp90 is required for bHLH transcription factors, MyoD and E12, to assume a DNA binding conformation (20, 156). This chaperoning was demonstrated with purified hsp90 in a cell-free system without ATP, and no binding of hsp90 to the bHLH protein could be detected. This is very different from the stable DR bHLH·hsp90 complex that was generated by the hsp90-based chaperone system of reticulocyte lysate (153). It is likely that activation of the DNA binding conformation of MyoD and E12 reflects an ATP-independent chaperoning such as that described for the refolding of denatured enzymes is *in vitro* (2, 157).

There is only one report of immunophilin association with the nonsteroid-receptor transcription factors. Carver and Bradfield (158) used the yeast two-hybrid system to screen for proteins interacting in a ligand-dependent manner with the DR. They cloned the cDNA for a 37-kDa protein called ARA9 that possessed an N-terminal region with 30% identity to a major portion of the PPIase domain of FKBP52 and a C-terminal domain with three TPRs. The Perdew lab simultaneously cloned a 43-kDa protein that they had demonstrated in the cross-linked DR·hsp90 heterocomplex (159) and identified an FKBP (G. Perdew, personal communication) that appears to be ARA9. Although Carver and Bradfield (158) found that binding of this immunophilin homolog to the DR was ligand-dependent *in vivo,* the binding was ligand-independent *in vitro.* The fact that ARA9 bound to the free DR as well as the hsp90-bound DR suggests that the steroid receptors may directly bind FKBPs, such as FKBP51, FKBP52 and the FKBP-like PP5. As Carver and Bradfield (158) note, this observation also suggests that the DR·ARA9 interaction can be maintained during the cytoplasmic-nuclear trafficking of the DR. Such sustained association would be consistent with the model described above in which the high-molecular-weight immunophilins play a role in targeting receptor trafficking (89, 139).

The p53 Tumor Suppressor. The tumor suppressor protein p53 is a transcription factor that is commonly found in mutated forms in tumor cells. Many of the p53 mutants have a prolonged half-life, and it is known that sustained binding of a protein to hsp90 often prolongs its half-life. Because the hsp90-binding antibiotic geldanamycin was known to destabilize several oncogene and proto-oncogene products, Blagosklonny *et al.* (160) treated cells with geldanamycin and found that the half-life and steady state level of the mutated p53 was greatly reduced whereas the wild type protein was unaffected. They then showed that mutated p53 translated in reticulocyte lysate formed a heterocomplex with hsp90 (25). Using a temperature-sensitive mutant of p53, Sepehrnia *et al.* (24) found that at the non-permissive temperature, the p53 mutant was localized in the cytoplasm and in a heterocomplex with hsp90, whereas at the permissive temperature, the p53 was nuclear and was not immunoadsorbed with hsp90. Thus, as for the GR, hsp90 affects both the stability and localization of the p53 mutants.

A major question that arises is whether wild-type p53 never associates with hsp90 or whether it associates with it in a weak and dynamic manner such that the association evades biochemical detection. It is a reasonable notion that hsp90 is involved in the normal chaperoning of wild-type p53 and that the p53 mutants are converted to a stable association with hsp90 similar to that seen with steroid receptors. The estrogen receptor (ER) provides a similar example. Chambraud *et al.* (161) showed that two regions of the ER, the HBD and the C-terminal end of the DNA binding domain, are required to demonstrate stable association with hsp90. Although ER HBD alone does not bind hsp90 tightly enough such that binding can be demonstrated by the usual biochemical techniques, it does confer hormonal control of physiological response onto chimeric proteins (162). The failure to show hsp90 binding by chimeras containing the ER HBD has caused some to erroneously conclude that hsp90 is not necessary for controlling hormone-dependent transcription by the ER (163). In fact, when rapid and gentle techniques of immunoadsorption are used, one can demonstrate that hsp90 is bound to a chimera containing only the HBD of the ER, and ER HBD·hsp90 heterocomplexes are formed *in vitro* by reticulocyte lysate (82). Importantly, Aumais *et al.* (164) have shown that a G400V mutation in the ER HBD yields a chimera that is stably bound to hsp90, rendering it hormone-dependent for DNA binding *in vitro* and more strongly dependent on estradiol for transcription than a chimera with the wild-type HBD. In a similar manner, the p53 mutants that form stable heterocomplexes with hsp90 may provide a fortuitous view into what is an otherwise biochemically undetectable role for hsp90 in the normal chaperoning of the wild-type p53.

Hepatitis B Virus Reverse Transcriptase. Reverse transcription in hepatitis B viruses is initiated by a protein-priming mechanism in which the virally encoded reverse transcriptase binds to a short RNA sequence (called ϵ) located at the 5′ end of the viral pregenomic RNA, and DNA synthesis is initiated using a tyrosine residue in the polymerase polypeptide as the primer. The complex between the reverse transcriptase and ϵ is transient, and cellular factors are required to stabilize the polymerase in a conformation that allows ϵ binding.

It was first shown that reverse transcriptase translated in the reticulocyte lysate system forms a stable complex with ϵ RNA and is active in the protein-priming reaction (165, 166). However, the protein-priming activity of the polymerase expressed in wheat germ extract was less than 2% of the enzyme translated in reticulocyte (23). A similar disparity had previously been noted with the GR where the receptor translated in reticulocyte lysate was converted to the steroid binding conformation, but the receptor translated in wheat germ extract was not (47). It was subsequently shown that GR·hsp90 complexes formed in wheat germ extract were unstable and that addition of purified p23 stabilized the GR·plant hsp90 complexes and the steroid binding activity that was generated by the plant hsp90-based chaperone system (73). Based on these observations with the GR, the presence of an activity after translation of a protein in reticulocyte lysate combined with the absence of activity after its translation in wheat germ extract have led investigators in several instances to ask whether there is a role for the hsp90-based chaperone system in generating the proper protein conformation (4).

In the case of the reverse transcriptase, Hu and Seeger (23) showed that the enzyme translated in reticulocyte was bound to hsp90. Antibodies against hsp90 blocked the formation of a complex between the polymerase and ϵ RNA,

and the hsp90-specific inhibitor geldanamycin inhibited the protein priming activity of polymerase translated in reticulocyte lysate (23). Apparently, the function of the hsp90-based chaperone system is to form a complex with hsp90 in which the polymerase is maintained in a unique conformation required for the initial priming reaction, a conformation that is different from that required for the subsequent elongation reaction (23). An interesting difference between the reverse transcriptase and the steroid receptors is that the polymerase does not appear to dissociate from hsp90 after binding to its ϵ RNA ligand (23).

Hsp90 Heterocomplexes with Protein Kinases

Since the discovery of the pp60$^{v\text{-}src}$·hsp90 complex in 1981 (26, 27), at least 13 additional protein kinase·hsp90 complexes have been identified (see Table I), and hsp90 is now thought to play a role in signal transduction from a variety of receptors located in the plasma membrane.

Some receptors for polypeptide ligands, such as those for insulin, epidermal growth factor (EGF), platelet-derived growth factor, and nerve growth factor, transduce their signals by activating the MAP (mitogen-activated protein) family of serine/threonine kinases (also called Erks for extracellular signal–regulated kinases). The receptors are tyrosine kinases that undergo ligand-induced autophosphorylation, an event that leads through binding of the factors Grb2 and Sos to the activation of Ras. Subsequent binding of Ras to the Raf-1 serine/threonine kinase leads to phosphorylation by Raf-1 of Mek (also called MAP kinase kinase), which then phosphorylates and activates MAP kinase. It is MAP kinase that travels to the nucleus and acts as the terminal effector in the signalling pathway by directly phosphorylating transcription factors that regulate gene expression. The receptor-mediated signalling system can be short circuited by the oncoprotein Src, which can activate Raf directly. Three components of this system, Src, Raf, and Mek, form stable complexes with hsp90.

Src, Raf, and Mek Bind hsp90. The first stable complex of any protein with hsp90 was noted by Brugge *et al.* (26) and Oppermann *et al.* (27) who reported that immunoadsorption of the viral oncoprotein pp60$^{v\text{-}src}$ (v-Src) from cytosol was accompanied by co-immunoadsorption of two cellular proteins of molecular weight 90,000 and 50,000. Other avian retroviral transforming proteins that were tyrosine-specific protein kinases were also found in heterocomplexes with the 90-kDa and 50-kDa proteins (for review, see Ref. 3). The heterocomplex with v-Src was formed when the kinase was translated, and v-Src remained in a cytosolic heterocomplex with the 90-kDa protein while it underwent transport to the cell membrane (167, 168). Although the function of the Src multiprotein complex still has not been defined, Courtneidge and Bishop (167) originally suggested that the cytoplasmic complex may be the vehicle by which pp60$^{v\text{-}src}$ reaches the plasma membrane.

In 1985, it became clear that the 90-kDa component of the Src heterocomplex and the 90-kDa component of steroid-receptor heterocomplexes were the same protein, hsp90 (11). The Src and steroid receptor complexes are biochemically very similar in that Src·hsp90·p50 complexes, like the receptor·hsp90·immunophilin complexes, are formed by the hsp90-based chaperone system of reticulocyte lysate (28); the complexes are destabilized by the same conditions, and they are both stabilized by molybdate, vanadate, and tungstate (169). The Raf and Mek components of the MAP kinase signalling system also exist in native complexes with hsp90 that can be formed *in vitro* by reticulocyte lysate (39–42). It has been shown for both Src (170) and Raf (39) that the catalytic domain of the kinase is sufficient for hsp90 binding.

Role for hsp90 in Signalling *via* MAP Kinase. Normally, only a small fraction (~5%) of the total cellular v-Src is in heterocomplex with hsp90 and p50 (26, 167), with the low percentage probably reflecting the portion of v-Src molecules that are in transit to the cell membrane at any time. However, with certain temperature-sensitive Src variants that are partially defective at cellular transformation, more than 90% of the Src molecules are recovered in stable heterocomplexes with hsp90 (170). This suggests that binding to hsp90 is in some way related to the transforming activity of Src.

Genetic observations made in yeast, where expression of v-Src produces growth arrest, support the conclusion that hsp90 is somehow critical for the function of v-Src. Xu and Lindquist (171) showed that a mutation that lowered the level of hsp90 expression in yeast relieved cell cycle arrest and rescued growth in cells expressing v-Src. Growth inhibition by v-Src was also reduced in yeast expressing a mutant Ydj1 protein (the yeast homolog of hsp40) that led to derepression of GR and ER function (76). A linkage between hsp90 requirements for steroid receptor activity and v-Src activity is also suggested by the fact that several point mutations in yeast hsp90 displayed both decreased GR stimulation of reporter gene expression and v-Src activity (172).

Genetic data from *Drosophila* also show that hsp90 is critical for signalling *via* MAP kinase. The sevenless receptor is a protein tyrosine kinase that is required for the differentiation of the R7 photoreceptor neuron during development of the compound eye of *Drosophila melanogaster.* Signalling from the sevenless receptor occurs *via* activation of the MAP kinase system (173). Cutforth and Rubin (33) have shown that signalling by the sevenless receptor is reduced by mutations in *E(sev)3A,* which is a member of the hsp90 family.

Geldanamycin. The antibiotics geldanamycin and herbimycin have been useful tools for studying the role of hsp90 in signalling *via* the MAP kinase system. These benzoquinone ansamycins were found to revert transformation by tyrosine kinase oncogenes, such as *src, yes, fps, abl,* and *erbB* (174, 175). Although geldanamycin was thought originally to inhibit the kinase activity of Src (176), Whitesell *et al.* showed that geldanamycin had a tumoricidal activity

unrelated to Src kinase inhibition (177), and instead, the antibiotic bound in a pharmacologically specific manner to hsp90 (44). The binding of geldanamycin to hsp90 leads to a block in Src·hsp90 heterocomplex assembly (44) and an increase in the rate of Src turnover (176).

Geldanamycin also inhibits the assembly of Raf·hsp90 complexes and markedly increases the rate of Raf turnover (42, 178). Smith *et al.* (179) showed that, in the presence of geldanamycin, progesterone receptor heterocomplex assembly is blocked at the normally transient PR·hsp90·p60·hsp70 intermediate complex (Step 2 product in Figure 1), and it was subsequently shown that Raf heterocomplex assembly is blocked at an identical intermediate stage of assembly (42). Importantly, geldanamycin inhibits signalling through the EGF receptor without affecting the EGF-mediated increase in the specific activity of Raf kinase (42). The inhibition of signalling through the MAP kinase system may be indirect in that geldanamycin increases the rate of degradation of Raf and perhaps other components in the signalling pathway (42).

Another level of geldanamycin effect could be on the turnover of the receptor itself. Geldanamycin and herbimycin have been reported to increase the turnover rate and decrease the cellular activity of transmembrane tyrosine kinase receptors, such as EGF, insulin-like growth factor, and insulin receptors, and $p185^{c\text{-}erbB\text{-}2}$ (180, 181). In these instances, the receptor-associated protein is the glucose-regulated protein GRP94, which is a member of the hsp90 family and also binds geldanamycin (182). In cells treated with geldanamycin, these receptors are degraded *via* an ubiquitin-dependent, 20S proteasome-mediated pathway (180, 181). The GR undergoes degradation by a similar proteasomal mechanism in geldanamycin-treated cells (45). Thus, one consequence of stable association of a protein with members of the hsp90 family is stabilization of the protein to ubiquitin-dependent proteasomal degradation, regardless of whether the hsp90-chaperoned protein is a transcription factor, a signalling protein kinase such as Src or Raf, or a transmembrane tyrosine kinase.

$p50^{cdc37}$. The p50 component of the protein kinase·hsp90 heterocomplexes is not a member of the immunophilin family. p50 was recently identified as the mammalian homolog of the yeast cell cycle control protein cdc37 (183, 184), and it is now called $p50^{cdc37}$. $p50^{cdc37}$ does not contain TPRs, and its binding to hsp90 is not competed by the TPR domains of immunophilins, but $p50^{cdc37}$ binding is competed by the p60 component of the hsp90-based heterocomplex assembly system (89). This suggests that the $p50^{cdc37}$ binding site on the surface of hsp90 lies close to the TPR binding site but does not include it. It is thought likely that $p50^{cdc37}$ and an immunophilin cannot be simultaneously bound to hsp90 (89), but this has not yet been established unequivocally.

Interestingly, $p50^{cdc37}$ is not present in native steroid receptor·hsp90 heterocomplexes (39, 185), and the native Raf·hsp90 heterocomplex contains $p50^{cdc37}$ but not FKBP52 (39, 186). This is consistent with the notion that the protein that is occupying the chaperone site on hsp90 (i.e., receptor or protein kinase) determines the composition of the heterocomplexes that are formed. As with the steroid receptors, the protein kinases can probably be in several different hsp90 heterocomplexes. For example, the immunoadsorbed Raf-1 heterocomplex binds [^{3}H]FK506 in a manner that is specific for an immunophilin and dependent upon Raf-1 binding to hsp90 (186). Thus, although the majority of Raf·hsp90 complexes contain $p50^{cdc37}$, some Raf·hsp90 complexes must contain an FKBP.

Genetic evidence suggests that $p50^{cdc37}$ is necessary for proper signalling by some protein tyrosine kinases *in vivo.* For example, mutations in cdc37 impair v-Src function in yeast (187), and mutations in *E(sev)3B,* a homolog of cdc37 in *Drosophila,* impair signalling by the sevenless receptor (33). The function of $p50^{cdc37}$ in protein kinase heterocomplexes, such as Src and Raf, is unknown. The cellular localization of p50 is consistent with the notion (39, 89) that it targets movement of Src and Raf to their sites of action. $p50^{cdc37}$ is concentrated in the perinuclear region of the cell from which it extends out on cytoskeletal fibrils to the cell periphery where some of it is localized to the inner surface of the plasma membrane (89). It is possible that FKBPs, PP5 and $p50,^{cdc37}$ play distinct roles in the shuttling of a variety of signalling molecules between the plasma membrane and the nucleus.

Heme-Regulated eIF2α Kinase. The regulation of the protein synthesis initiation factor eIF2 by hsp90 may reflect a ligand-dependent mechanism akin to that of steroid receptors. In reticulocyte lysate that is deficient in heme, protein synthesis is inhibited due to activation of a serine/threonine protein kinase, called heme-regulated eIF2α kinase or HRI (heme regulated inhibitor), that phosphorylates eIF2 on its α subunit. Phosphorylated eIF2 forms an inactive complex with eIF2B, a factor that is necessary for recycling eIF2 to its active GTP-bound form. Because eIF2B is in lower abundance than eIF2, when the heme concentration is low, all of the eIF2B becomes bound in an inactive complex, and protein synthesis is inhibited.

Hardesty and his co-workers showed that eIF2α kinase co-purified with hsp90 and that hsp90 stimulated its ability to inhibit eIF2 (34, 188). Data reported by Matts and Hurst (35) suggested a model of regulation in which the hsp90-bound eIF2α kinase is inactive in the presence of heme, but when heme levels become low, hsp90 dissociates, and the kinase is activated. How the lack of heme initiates the process to yield an active, dissociated form of eIF2α kinase is unclear, and the actual site of heme action (e.g., eIF2α·hsp90 complex) is unclear. Interestingly, the eIF2α kinase·hsp90 heterocomplex is similar to steroid receptor heterocomplexes in that hsp70 and FKBP52 are also present (189).

Summary

From the study of steroid receptor heterocomplexes has come the discovery and reconstitution of the hsp90-based chaperone system. We have, as yet, very little mechanistic understanding of how this system carries out the folding/unfolding process. However, it is clear that the system is ubiquitous and basic to the biology of all eukaryotic cells, with apparently total conservation of function on the part of chaperones from the animal and plant kingdoms. The availability of geldanamycin to probe for hsp90-dependent proteins and protein activities should facilitate their rapid discovery, and it is likely that a review of this field a decade hence will see a great expansion in the number of these proteins.

The high-molecular-weight immunophilins are also a unique discovery emanating from the study of the structure of untransformed receptor heterocomplexes. Again, these proteins appear to be ubiquitously expressed in eukaryotic cells, but their fundamental reason for being is not yet clear. It is likely that more immunophilins will be found, and it is possible that there are other, as yet undiscovered, proteins that bind to the $p50^{cdc37}$ binding site on hsp90. Because the immunophilins bind to a receptor·hsp90 heterocomplex in a reversible manner, a single receptor heterocomplex can vary its composition dynamically in time. The protein kinase·hsp90 heterocomplexes likely change in a similar dynamic manner. The dynamic nature of the complexes may be necessary if these hsp90-associated proteins are involved in the targeted trafficking of proteins through the cytoplasm.

For the most part this review has emphasized biochemical studies in cell-free systems, and such studies have been the source of most of the new concepts regarding the hsp90-based chaperone system. Equally important are the genetic studies confirming the role of this system in signalling from receptors in cells. These genetic studies are the subject of an excellent current review by Picard (190).

Preparation of this review and the author's work reported herein were supported by National Institutes of Health Grants CA28010 and DK31573.

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