Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins

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Summary

Heat shock proteins interact with multiple key components of signaling pathways that regulate growth and development. The molecular relationships between heat shock proteins, various signaling proteins and partner proteins appear to be critical for the normal function of signal transduction pathways. The relative levels of these proteins may be important, as too little or too much Hsp70 or Hsp90 can result in aberrant growth control, developmental malformations and cell death. Although the functions of heat shock proteins as molecular chaperones have been well characterized, their complementary role as a 'stress-induced' proteins to monitor changes and alter the biochemical environment of the cell remains elusive. Genetic and molecular interactions between heat shock proteins, their co-chaperones and components of signaling pathways suggest that crosstalk between these proteins can regulate proliferation and development by preventing or enhancing cell growth and cell death as the levels of heat shock proteins vary in response to environmental stress or disease.

Key words: Signal transduction, Hsp70, Hsp90, Bag1, Nuclear hormone receptors, Kinases, Molecular chaperones, Protein folding, Protein aggregation

Too much or too little is not good

The role of heat shock proteins (HSPs) in protein folding, transport and complex formation has been studied extensively (Bukau and Horwich, 1998; Hartl, 1996). Their roles in signal transduction have been established from observations that Hsp90 and Hsp70 are associated with a number of signaling molecules, including v-Src, Raf1, Akt and steroid receptors (Dittmar et al., 1998; Sato et al., 2000; Xu and Lindquist, 1993). Decreasing the levels of functional Hsp90 in *Drosophila* by genetic mutation or by treatment with an Hsp90 inhibitor geldanamycin causes developmental abnormalities (Rutherford and Lindquist, 1998). Likewise, increasing the levels of Hsp70, by gene transfer mediated overexpression or by heat shock, has growth-inhibitory effects on mammalian tissue culture cells and in *Drosophila* salivary gland cells, whereas expression of a dominant-negative form of Hsp70 causes developmental defects in *Drosophila* (Elefant and Palter, 1999; Feder et al., 1992). However, what remains less well understood is whether this is a general strategy used by the cell to link specific signaling pathways with cell-stress-sensing events.

Interestingly, cells that have lost their ability to regulate cell growth, such as tumor cells, often express high levels of multiple HSPs compared with their normal parental cells (Jaattela, 1999). Depletion of Hsp90 by geldanamycin or of Hsp70 by anti-sense methodology in transformed cells, but not in their non-transformed counterparts, causes either arrest of cell growth or cell death (Nylandsted et al., 2000; Whitesell et al., 1994). Tumor cells appear to be dependent on increased levels of HSPs, although why this is beneficial has yet to be clearly established. One possibility is the ability of chaperones to suppress and buffer mutations that accumulate during the transformation process, which could promote cell viability and even enhanced cell growth of otherwise mutant cells. This is exemplified by the relationship between p53, Hsp70 and Hsp90, where mutant forms of p53, but not wild-type p53, depend on Hsp70 and Hsp90 for normal level and function (Blagosklonny et al., 1996; Pinhasi-Kimhi et al., 1986).

Hsp70 and Hsp90 are heat shock proteins

When cells are exposed to conditions of proteotoxic stress - for example heat shock - the expression of HSPs, including members of the Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small HSP families, is induced (Morimoto, 1998; Parsell and Lindquist, 1993). HSPs have a critical role in the recovery of cells from stress and in cytoprotection, guarding cells from subsequent insults. They protect stressed cells by their ability to recognize nascent polypeptides, unstructured regions of proteins and exposed hydrophobic stretches of amino acids. In doing so, chaperones hold, translocate or refold stress-denatured proteins and prevent their irreversible aggregation with other proteins in the cell (Bukau and Horwich, 1998; Morimoto, 1998; Parsell and Lindquist, 1993).
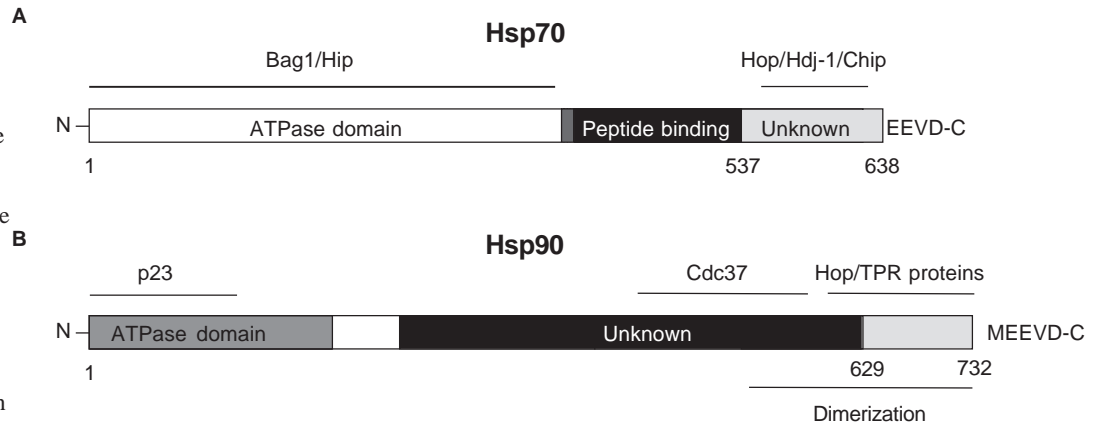
In addition to their roles in protecting cells from stress, nearly all HSPs are constitutively expressed under normal growth conditions, where they function to maintain protein homeostasis by regulating protein folding quality control. The chaperone activities of heat shock proteins enable folding of newly synthesized proteins and assist protein translocation across intracellular membranes (Hartl and Hayer-Hartl, 2002; Neupert, 1997).

Here, we focus on the activities of two abundantly expressed and highly conserved heat shock proteins: Hsp70 and Hsp90. The levels of human Hsp70 and Hsp90 vary among primary

Fig. 1. Schematic and linear representation of the domain structure and co-chaperone-binding sites of Hsp70 and Hsp90. The amino-acid residue numbers, domains and co-chaperone-binding sites are indicated. (A) Domain structure of human Hsp70. The C-terminal EEVD motif is characteristic for cytosolic Hsp70s and is involved in binding of TPR-domain-containing proteins.
(B) Domain structure of human Hsp90α. The C-terminal MEEVD motif is involved in binding of TPR-domain-containing proteins. Binding sites of co-chaperones on Hsp90 are a composite of sites of interaction for mammalian and yeast Hsp90 [adapted from previous reports (Chen and Smith, 1998; Grammatikakis et al., 1999; Young et al., 2001)].

and transformed cell lines and range from 10 μM-100 μM for Hsp70 and 10 μM to 150 μM for Hsp90 (C. Schmidt and R.I.M., unpublished). Hsp70 and Hsp90 represent two different classes of molecular chaperone. Whereas Hsp70 holds unfolded substrates in an intermediately folded state to prevent irreversible aggregation and catalyzes the refolding of unfolded substrates in an energy- and co-chaperone-dependent reaction, Hsp90 appears to interact with intermediately folded proteins and to prevent their aggregation but lacks the ability of Hsp70 to refold denatured proteins. In vitro chaperone assays using chemically denatured proteins have shown that Hsp90 is highly efficient in preventing protein misfolding and that the Hsp70 chaperone machinery is required to fold the Hsp90-released intermediates to the native state (Freeman and Morimoto, 1996; Jakob et al., 1995; Wiech et al., 1992).

Biochemical activities of Hsp70

Hsp70 interacts with co-chaperones through the N-terminal ATPase domain and with substrates at the C-terminal substrate-binding domain (Fig. 1a). Binding of exposed stretches of hydrophobic residues in unfolded or partially unfolded proteins is regulated by ATP-hydrolysis-induced conformational changes in the ATPase domain of Hsp70, which is stimulated by the co-chaperone Hsp40 (Hdj-1). Release of substrates requires the binding of ATP to Hsp70, after which the substrates either enter a new cycle of binding and release or fold into their native conformation (Freeman et al., 1995; Minami et al., 1996; Szabo et al., 1994).

Co-chaperone interactions can influence the Hsp70-substrate binding-and-release cycle by stimulating, inhibiting or altering the trafficking of Hsp70-interacting substrates. Hip binds to the ATPase domain and increases the chaperone activity of Hsp70 by stabilizing the ADP state, which is the substrate-bound state of Hsp70 (Fig. 1a) (Hohfeld et al., 1995). Bag1, by contrast, inhibits the chaperone activity of Hsp70 in part by accelerating nucleotide exchange, which affects the premature release of the unfolded substrate (Bimston et al., 1998; Hohfeld and Jentsch, 1997; Takayama et al., 1997; Zeiner et al., 1997). Hip and Bag1 bind to Hsp70 at the same site on the ATPase domain and directly compete to influence Hsp70 chaperone activity (Fig. 1a) (Hohfeld and Jentsch, 1997; Nollen et al., 2001). The

biological role of these co-chaperones in the regulation of Hsp70, however, is not well understood. In part, this is because the levels of Hip and Bag1 are approximately 1% of Hsp70, and biochemical studies have shown that Bag1, for example, affects the Hsp70 chaperone activity in a 1:1 molar ratio (Nollen et al., 2000; Takayama et al., 1997). This would indicate that Bag1 influences only a fraction of the Hsp70 molecules in the cell and therefore is unlikely to be an essential co-chaperone of Hsp70. Bag-1 and Hip also interact with other proteins in the cell. Bag1, for example, interacts with and influences the function of many key components of cell death and signal transduction pathways, including the anti-apoptotic protein Bcl-2 and the growth regulator Raf-1 (Takayama and Reed, 2001; Wang et al., 1996). We propose that the regulation of Bag1 function by Hsp70 serves as a checkpoint to regulate growth and death when the levels of Hsp70 in the cell rise in response to stress (Song et al., 2001).

Biochemical activities of Hsp90

Hsp90 substrates are less well understood; however, genetic data support a role for Hsp90 interaction with signaling molecules and components of cell death pathways (Pratt and Toft, 1997). A consensus substrate recognition sequence for Hsp90 has yet to be identified, and Hsp90 is not required for de novo protein synthesis (Nathan et al., 1997). Common to many of the Hsp90 partner proteins is that they are regulated, either negatively or positively, by chaperone interaction. The substrates also have in common the fact that they are short lived and prone to alternative conformations. Binding of Hsp90 to these substrates prevents degradation and changes their conformations, as suggested by their altered proteolytic sensitivity, enzymatic activity, and, in the case of hormone receptors, their ability to bind ligands (Pratt and Toft, 1997).

The biochemical mechanism of Hsp90-substrate interactions is currently a topic of active investigation, and, to date, has only partially been characterized. Although Hsp90-substrate binding is ATP independent, substrate release requires ATP hydrolysis (Panaretou et al., 1998; Prodromou et al., 1997). The Hsp90-substrate binding-and-release cycle is regulated by sequential interactions of Hsp90 with the co-chaperones Hop and p23. Hop is a tetratricopeptide repeat (TPR)-domain-

Table 1. Examples of Hsp90 and Hsp70 co-chaperones, interacting molecules and cellular function

Chaperone	Cellular function	Co-chaperone	Preferred interaction partner	References
Hsp90	Signal transduction	Cdc37	Kinases	Cutforth and Rubin, 1994; Gerber et al., 1995; Grammatikakis et al., 1999; Kimura et al., 1997; Perdew et al., 1997; Schutz et al., 1997
	Hormone response	PP5 (TPR)	Glucocorticoid receptor	Silverstein et al., 1997
		Immunophilins (TPR):	Hormone receptors:	
		Cyp40	Oestrogen receptor	Silverstein et al., 1997
		FKBP52	Steroid receptor	Davies et al., 2002; Silverstein et al., 1997
		FKBP51	Progesteron/ glucocorticoid receptor	Davies et al., 2002; Silverstein et al., 1997
	Cell death	Unknown	Apaf-1	Pandey et al., 2000
	Stress response	Unknown	HSF-1	Zou et al., 1998
	Complex assembly	p23	Chaperone complex	Young et al., 1998
		Hop	Chaperone complex	Prapapanich et al., 1996
Hsp70	Signal transduction	Bag1	Raf-1 kinase	Wang et al., 1996
		Bag1	Growth factor receptors	Bardelli et al., 1996
	Hormone response	Bag1	Hormone receptors	Zeiner and Gehring, 1995
		Hip	Steroid receptor	Prapapanich et al., 1996
	Cell death	Bag1	Bcl-2	Takayama et al., 1995
		Unknown	Apaf-1	Beere et al., 2000
	Stress response	Unknown	HSF-1	Abravaya et al., 1992
	Complex assembly	Hop	Chaperone complex	Chen and Smith, 1998
		Hdj-1	Chaperone complex	Caplan et al., 1995; Dittmar et al., 1998; Kosano et al., 1998

containing protein that binds to the C-terminal domain of Hsp90. Binding of Hop induces a conformational change in the ATPase domain of Hsp90 that inhibits the ATPase activity (Prodromou et al., 1999). Dissociation of the Hop and Hsp70 from Hsp90, by an as yet unknown mechanism, results in a conformational change in the ATPase domain that enables binding of ATP and a transient dimerization of the N-termini of Hsp90 molecules, whereas their C-terminal domains remain dimerized. In turn, this allows for association with p23 and members of the immunophilin family, followed by ATP hydrolysis and opening of the molecular clamp formed by the N-terminal ends of the Hsp90 dimer to release the substrate. The Hsp90-specific inhibitor geldanamycin competes with ATP at the ATP-binding site and thus prevents completion of the interaction cycle by interfering with the association of p23 and ATP hydrolysis (Prodromou et al., 2000; Prodromou et al., 1999; Young and Hartl, 2000). Hsp90 also associates with other co-chaperones besides Hop and p23, including other TPR-domain-containing proteins and Cdc37 (Table 1). Many of these co-chaperones compete for binding to the C-terminal domain of Hsp90 (Fig. 1b), which suggests that co-chaperones might play a role in targeting Hsp90 to specific substrates.

Interaction between chaperones and signaling molecules

The ability of a cell to know whether to grow, divide, differentiate or die depends upon extracellular signals and the ability to properly recognize and respond to these signals. The cell may receive these extracellular signals in different forms such as soluble hormones, small peptides, or proteins attached to neighboring cells. Cellular receptors receiving these signals transmit the extracellular information to the nucleus through cascades of protein-protein interactions and biochemical reactions. Chaperones of the Hsp90 and Hsp70 family and their co-chaperones interact with a growing number of signaling molecules, including nuclear hormone receptors, tyrosine- and serine/threonine kinases, cell cycle regulators and cell death

regulators (Table 1). Although the functions of many signaling molecules have been studied in detail, the significance of their interaction with chaperones and co-chaperones remains obscure.
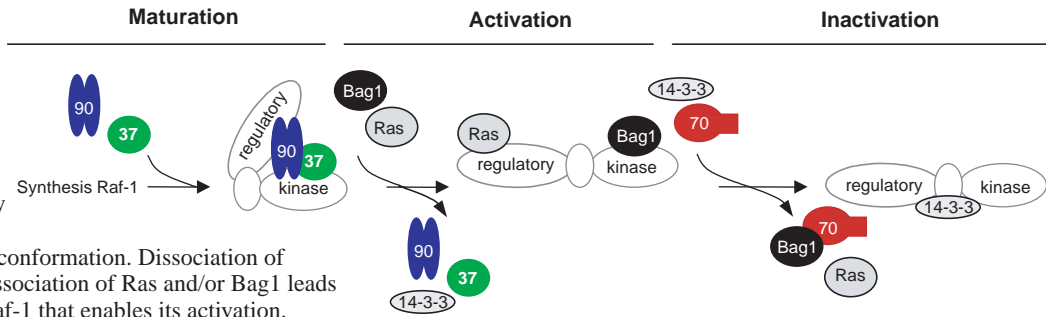
Below we describe two examples of signaling routes in which chaperones of the Hsp90 and Hsp70 families play a role: the Ras/Raf-1 signal transduction pathway and the nuclear hormone aporeceptor complex assembly. Along with these examples we describe what is known about the role of chaperones and co-chaperones in these processes.

Chaperones and the Ras/Raf-1 signal transduction pathway

The Ras/Raf-1 signaling pathway has an important role in proliferation, cell differentiation, growth arrest and death (Campbell et al., 1998; Kolch, 2000). In response to extracellular signals, Ras is activated via the activities of membrane-bound receptors and a set of modulators. Activated Ras targets a Raf-1 heteromeric complex to the membrane, where Raf-1 is activated by a myriad of activating proteins in a process that is poorly understood. Upon activation, Raf-1 kinase initiates a cascade of downstream reactions ultimately leading to phosphorylation of the 'extracellular signal regulated kinases' ERK1 and ERK2 that regulate transcription factors in the nucleus. The response depends on the cell type and the duration and strength of the external stimulus (Campbell et al., 1998; Kolch, 2000; Sternberg and Alberola-Ila, 1998).

Hsp90 has been found in association with many components and regulators of the Ras/Raf-1 pathway, including Raf-1, Ksr-1, Akt and Src (Kolch, 2000; Sato et al., 2000; Stewart et al., 1999; Whitesell et al., 1994). Genetic studies in *Drosophila* and biochemical studies in mammalian cells have demonstrated that interactions between Hsp90 and Raf-1 are essential for Raf-1 activation. Mutations in *Drosophila* Hsp90 that decrease the interaction between Raf-1 and Hsp90 suppress Raf-1 gain-of-function mutants, which affect eye

Fig. 2. A model for chaperone and co-chaperone interactions with Raf-1. Chaperones and co-chaperones play a role in the maturation, activation and inactivation of Raf-1. During maturation Cdc37 (37) and Hsp90 (90) bind to the regulatory and kinase domains of Raf-1 (in white) and keep it in an inactive conformation. Dissociation of Cdc37 and Hsp90 followed by association of Ras and/or Bag1 leads to a conformational change of Raf-1 that enables its activation. Sequestration of Bag1 by Hsp70 (70) and association of 14-3-3 leads to the inactivation of Raf-1. Raf-1 co-associating and regulatory proteins other than Ras, chaperones or co-chaperones are omitted from the model for the sake of simplicity (see text for details and References).

development. The same mutations in Hsp90 that suppress activated Raf-1 also reduce the biochemical activity of activated Raf-1 (Cutforth and Rubin, 1994; van der Straten et al., 1997).

Similarly, prolonged exposure of mammalian cells to the Hsp90 inhibitor geldanamycin dissociates Raf-1-Hsp90 complexes, resulting in decreased Raf-1 activity owing to enhanced degradation of the Raf-1 protein (Schneider et al., 1996; Stancato et al., 1997). By contrast, short exposures to geldanamycin leads to Raf-1 activation, suggesting that the transient release of Hsp90 is essential for activation. One way to interpret the duration-dependent effects of exposure to geldanamycin is that Hsp90 is required for maturation and maintenance of the stability of Raf-1 but is released for activation of Raf-1 by other regulators. Hsp90 release may even be essential for kinase activation; this has been suggested by analogous studies on PKR, an interferon-induced serine/threonine kinase. Hsp90 binding, although required for adoption of a conformation primed for activation, inhibits PKR activity by binding to the kinase domain and regulatory domain simultaneously (Donze et al., 2001). Deletion of the N-terminal domain of Raf-1 results in constitutive activation, suggesting that a conformational change of Raf-1 is required. Altogether, these studies suggest that binding of Hsp90 is essential for maturation of Raf-1 but that it needs to be released for activation (Fig. 2).

Hsp90 binding to Raf-1 also requires the interaction with the Hsp90 co-chaperone Cdc37. Genetic studies in *Drosophila* and gene-transfer-mediated overexpression studies in mammalian cells have shown that Cdc37 is essential for Raf-1 activation. Mutations in Cdc37 have effects on *Drosophila* eye development owing to impaired signaling through the MAPK signaling pathway, whereas overexpression of Cdc37 leads to dose-dependent activation of the wild-type but not a constitutively active form (Y340D) of Raf-1 (Cutforth and Rubin, 1994; Grammatikakis et al., 1999). Expression of a C-terminal deletion mutant of Cdc37 that can no longer bind to Hsp90 inhibits activation of Raf-1 by Src and Ras, indicating that an interaction between Cdc37 and Hsp90 is essential (Grammatikakis et al., 1999). On the basis of these studies, Cdc37-Hsp90 complexes have been suggested to potentiate Raf-1 activation by making Raf-1 accessible to activation by tyrosine kinases and other regulatory molecules that promote phophorylation.

Additionally, the Hsp70 co-chaperone Bag1 has been shown to activate Raf-1 through interaction with its kinase domain

(Wang et al., 1996). Binding and activation by Bag1 requires the C-terminal domain of Bag1, which is also necessary for binding to Hsp70, but does not require the N-terminal ubiquitin-like domain (Wang et al., 1996). Activation by Bag1 can bypass the effects of overexpression of a dominant-negative form of Ras1, indicating that Bag1 acts independently of Ras (Song et al., 2001). In contrast to Cdc37 and Hsp90, it is not known whether Bag1 is essential for Raf-1 activation and through what mechanism Bag1 activates Raf-1. On the basis of mutational studies and the proposed mechanisms of activation by other interaction partners, Bag1 could activate Raf-1 in various ways: (1) recruitment of Raf-1 to the membrane, similar to Ras activation of Raf-1; (2) changing the conformation of the regulatory loop, as proposed for phosphorylation mutants of Raf-1 that are independent of Ras for their activation; or (3) stabilization of the active conformation (Chong et al., 2001).

Alternatively, activation of Raf-1 may require release of Hsp90. Therefore, it is possible that Bag1 binding could either displace or replace Hsp90 to stabilize the active conformation of Raf-1 (Fig. 3). Consistent with this suggestion, naturally occurring variations in the expression levels of Hsp70 may serve to inactivate Raf-1 when Hsp70 forms complexes with Bag1, thus preventing its interaction with Raf-1 (Song et al., 2001). Altogether, chaperones and co-chaperones are essential for the maturation, stabilization and activation of the Raf-1 kinase. Although their biochemical roles in these processes remain to be further characterized, these may be similar to their well established roles in steroid aporeceptor complex formation as described below.

Chaperones and nuclear hormone aporeceptor complex assembly

Nuclear hormone receptors function as transcriptional regulators that are activated in response to binding of their specific hormone ligand. Multiple studies have shown that interactions between Hsp90 and Hsp70 and their respective co-chaperones is essential for activation of the nuclear hormone receptors (Arbeitman and Hogness, 2000; Picard et al., 1990; Pratt and Toft, 1997). The requirement for Hsp70 in receptor activation has recently been shown genetically for the ecdysone receptor in *Drosophila* (Arbeitman and Hogness, 2000).

Most of our knowledge of interactions between chaperones and nuclear receptors comes from biochemical studies on the
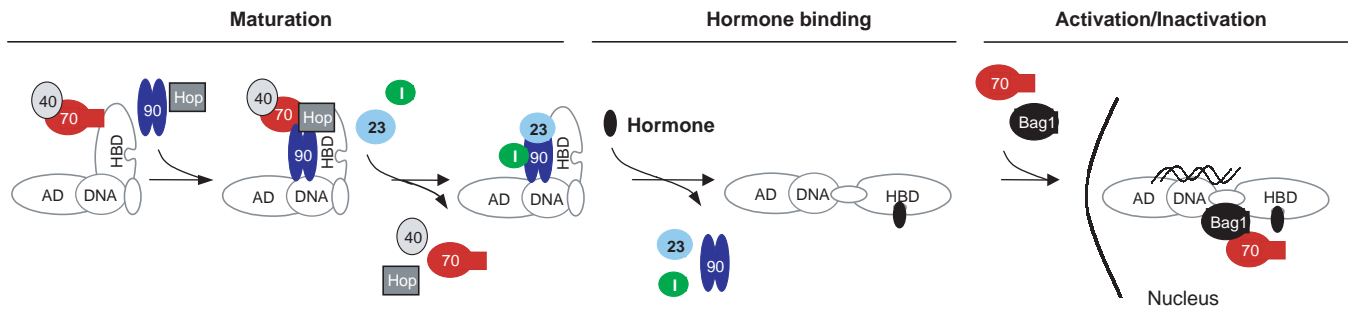
Fig. 3. A model for chaperone and co-chaperone interactions with nuclear hormone receptors. Chaperones and co-chaperones play a role in the maturation and inactivation of nuclear hormone receptors. During maturation, Hsp70 (70) and Hsp(40) bind to the hormone-binding domain (HBD) of the receptor (in white), which is followed by the association of Hop and Hsp90 (90). Maturation of the aporeceptor complex is completed by dissociation of Hsp40, Hsp70 and Hop followed by association of p23 (23) and one of the immunophilins (I). Hormone binding and dissociation of Hsp90, p23 and the immunophilin changes the conformation of the receptor, which then translocates to the nucleus and activates transcription. Bag1 can associate with the activated receptor and inhibit the activities of the receptor for which it requires its Hsp70-binding domain. AD, activation domain; DB, DNA-binding domain (see text for References).

glucocorticoid and progesterone receptors. In an inactive conformation, these receptors are in a heteromeric complex with Hsp90 and Hsp90-associating proteins. Before receptors are bound to Hsp90, they go through several steps of chaperone and co-chaperone interactions, which have also been shown to be essential by genetic studies in yeast (Chang and Lindquist, 1994; Chang et al., 1997; Dittmar et al., 1998; Duina et al., 1996). Initially the steroid aporeceptor interacts with Hsp70 and Hsp40. From this Hsp70-bound state, the aporeceptor is transferred to Hsp90 by the Hsp70- and Hsp90-binding co-chaperone Hop (Chen and Smith, 1998). Release of Hop, ATP binding and binding of the Hsp90 co-chaperone p23 then leads to the formation of the final aporeceptor complex that contains an Hsp90 dimer, p23 and immunophilins (Fig. 3) (Pratt and Toft, 1997).

Interaction with chaperones has been suggested to be required for stabilization of the aporeceptor conformation. Inactivation of Hsp90 by geldanamycin, for example, leads to an increase in protease sensitivity and degradation of glucocorticoid and progesterone receptors. Upon association of hormone with the aporeceptor complex, the chaperone complex is dissociated, and the receptor conformation transitions from a labile, open structure to the stable compact DNA-binding state that can exist independently of chaperone complexes (Pratt and Toft, 1997).

In addition to Hsp70 and Hsp90 complexes, other proteins, including the Hsp70-co-chaperones Hip and Bag1, have been found associated with hormone receptors (Prapapanich et al., 1996; Zeiner and Gehring, 1995). The role of Hip in receptor function, apart from its interaction with the aporeceptor complex, has not been well characterized. More is known about the role of Bag1 with respect to its interaction with hormone receptors. Bag1, also identified as RAP46 (for receptor associated protein 46), interacts with many nuclear hormone receptors, including the glucocorticoid receptor, the estrogen receptor and retinoic acid receptor (Liu et al., 1998; Zeiner and Gehring, 1995). Unlike Hsp70 and Hsp90 complexes, which bind to the inactive receptor and are released after activation, Bag1 appears to bind only to the activated receptors and after release of HSP complexes (Zeiner and Gehring, 1995). Overexpression studies in mammalian cells indicate that Bag1 co-migrates with the bound receptor to the nucleus and negatively regulates DNA binding and transactivation by the glucocorticoid receptor and prevents glucocorticoid-induced apoptosis (Kullmann et al., 1998; Schneikert et al., 1999). This activity is specific for two of the four isoforms of Bag1, the 46 kDa and the 50 kDa isoforms, which contain an N-terminal domain with a repeated [EEX4] motif and are expressed in the nucleus of mammalian cells. In addition, it requires the C-terminal Bag1 domain that is involved in binding to Hsp70, which suggests a role for Hsp70 in at least two steps of hormone receptor function (Schneikert et al., 2000). In complex with Hsp40 and Hop, Hsp70 functions in the cytosol to regulate aporeceptor complex formation, and together with Bag1 in the nucleus, it has a role in downregulation of the activated receptor (Schneikert et al., 2000). Taken together, these observations indicate that chaperone and co-chaperone interactions with hormone receptor are involved in both positive and negative regulation of hormone receptor activities in a temporally and spatially restricted manner.

In addition to components of the Ras/Raf-1 signaling pathway and hormone receptors, a wide variety of other molecules interact with Hsp70 and Hsp90 chaperone complexes, including the heat shock transcription factor, Apaf-1, CFTR and Hepatitis B viral reverse transcriptase (Abravaya et al., 1992; Beere et al., 2000; Hu and Seeger, 1996; Loo et al., 1998; Pandey et al., 2000; Zou et al., 1998). Although little is known about the molecular regulation and role of chaperones in these processes, mechanisms similar to the ones used for Raf-1 and hormone receptor regulation may be used, perhaps in collaboration with additional, yet to be identified co-chaperones.

Perspectives: consequences of variations in levels of chaperones and co-chaperones

As exemplified above for Raf-1 kinase and nuclear hormone receptors, the specificity of interactions between HSPs and substrates can be determined by co-chaperones. The levels and relative abundances of HSPs and the various co-chaperones vary widely among cells and tissues. This variation may provide each cell with a tailored chaperone network to support a cell-specific response to combinations of intra- and extracellular signals.

Several physiological, pathophysiological and environmental conditions, including development, aging, fever and several neurodegenerative diseases often associated with the accumulation of unfolded or misfolded proteins result in elevated expression of all HSPs and many but not all co-chaperones (Morimoto, 1998). Under such conditions, it may be less important that a particular unfolded polypeptide is associated with a specific chaperone; rather it is the conserved 'holding' function of chaperones that is essential, with triage decisions on the fate of these substrates being determined later during recovery.

As a consequence of these changes, however, the equilibrium between substrates, HSPs and co-chaperones is likely to be disturbed, which has potentially profound consequences for the phenotype of the cell. Changes in the abundance and relative levels of chaperones and co-chaperones could result in novel combinations of HSPs, which, in turn, could redirect information flow through the intracellular pathways and change the overall response to signals. Whereas some pathways may become favored because of an increase in the level of a particular co-chaperone that is specifically required for its regulation, other pathways might be suppressed or acquire constitutive activation by the lack of critical chaperone components. The overall effect of changes in chaperone or co-chaperone levels on cellular and organismal phenotypes probably depends on which chaperone or co-chaperone is affected. For example, changes in the levels of Hsp90 by exposure of cells or organisms to geldanamycin on in *Drosophila* by altering gene dosage, and Hsp70 by mutation or overexpression, have pleiotropic and often more severe consequences than do changes in the leels of a co-chaperone that is specific for a subset of substrates, such as Cdc37, which preferentially interacts with kinases. One example of a change in signaling as a consquence of altered levels of HSPs is the inhibition of the Ras/Raf-1 signaling pathway in tissue culture cells when the levels of Hsp70 increase in response to stress. The increased levels of Hsp70 sequester Bag1, which disrupts the stimulatory properties of Bag1 on Raf-1, which then results in cell growth arrest (Song et al., 2001).

HSPs have co-evolved as integral components of signal transduction networks, in which they can function in the maturation, activation and inactivation of signaling molecules. Their involvement in a particular pathway within the network is determined by the availability and relative abundance of partner-specific co-chaperones, which will influence, in a cell-type-specific manner the natural response to physiological intracellular and extracellular signals. Consequently, we suggest that altered levels of HSPs and co-chaperones in response to stress or disease states alters how organisms integrate and respond to the flow of their normal physiological signals. Future studies in multicellular model systems will help to elucidate with greater detail the molecular basis for the pervasive role of molecular chaperones in organismal development and disease and how they respond to altered chaperone and co-chaperone levels associated with fluctuating environmental conditions and disease.

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