MINIREVIEW / MINISYNTHÈSE

Hsp90: a chaperone for protein folding and gene regulation[1]

Rongmin Zhao and Walid A. Houry

Abstract: Molecular chaperones are essential components of a quality control machinery present in the cell. They can either aid in the folding and maintenance of newly translated proteins, or they can lead to the degradation of misfolded and destabilized proteins. Hsp90 is a key member of this machinery. It is a ubiquitous molecular chaperone that is found in eubacteria and all branches of eukarya. It plays a central role in cellular signaling since it is essential for maintaining the activity of several signaling proteins, including steroid hormone receptors and protein kinases. Hsp90 is currently a novel anticancer drug target since it is overexpressed in some cancer cells. The chaperone typically functions as part of large complexes, which include other chaperones and essential cofactors that regulate its function. It is thought that different cofactors target Hsp90 to different sets of substrates. However, the mechanism of Hsp90 function remains poorly understood. As part of an effort to elucidate the Hsp90 chaperone network, we carried out a large-scale proteomics study to identify physical and genetic interactors of the chaperone. We identified 2 highly conserved novel Hsp90 cofactors, termed Tah1 and Pih1, that bind to the chaperone and that also associate physically and functionally with the essential DNA helicases Rvb1 and Rvb2. These helicases are key components of the chromatin remodeling complexes Ino80 and SWR-C. Tah1 and Pih1 seem to represent a novel class of Hsp90 cofactors that allow the chaperone to indirectly affect gene regulation in the cell in addition to its ability to directly promote protein folding. In this review, we provide an overview of Hsp90 structure and function, and we discuss the literature that links the chaperone activity to gene regulation.

Key words: Hsp90, chaperone, cochaperone, gene regulation, protein folding.

Résumé : Les chaperons moléculaires sont des composantes essentielles de la machinerie de contrôle de qualité présente dans la cellule. Elles peuvent soit aider au repliement et au maintien des protéines nouvellement transcrites, ou diriger la dégradation de protéines mal repliées ou déstabilisées. Hsp90 est un membre clé de cette machinerie. Il s'agit d'un chaperon moléculaire ubiquiste trouvé dans les eubactéries et dans toutes les branches eucaryotes. Elle joue un rôle central dans la signalisation moléculaire car elle est essentielle au maintien de l'activité de plusieurs protéines signalétiques y compris les récepteurs d'hormones stéroïdiennes et les protéine-kinases. Hsp90 est maintenant une nouvelle cible pour les agents anti-cancéreux car elle est surexprimée dans certains types de cancer. Normalement, le chaperon fonctionne au sein de larges complexes qui comprennent d'autres chaperons ainsi que les co-facteurs essentiels qui régulent leur fonction. On croit que différents co-facteurs ciblent Hsp90 vers différents groupes de substrats. Cependant, le mécanisme de la fonction de Hsp90 reste peu compris. Comme contribution à l'effort visant à élucider le réseau de chaperons agissant de concert avec Hsp90, nous avons entrepris une étude protéomique à large spectre afin d'identifier les partenaires génétiques et physiques de ce chaperon. Nous avons identifié de nouveaux co-facteurs de Hsp90 hautement conservés, appelés Tah1 et Pih1, qui lient le chaperon et qui peuvent aussi s'associer physiquement et fonctionnellement avec les hélicases d'ADN Rvb1 et Rvb2. Ces hélicases sont des composantes clé des complexes de remodelage de chromatine Ino80 et SWR-C. Tah1 et Pih1 semblent représenter une nouvelle classe de co-facteurs de Hsp90 qui permettent au chaperon d'affecter indirectement la régulation génique de la cellule, ceci s'ajoutant à sa capacité de promouvoir directement le repliement des protéines. Dans cette revue, nous présentons une vue d'ensemble de la structure et de la fonction de Hsp90 et discutons des éléments tirés de la littérature qui lient l'activité chaperon à la régulation de gènes.

Mots clés : Hsp90, chaperon moléculaire, cochaperon, régulation du gène, repliement des protéines.

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R. Zhao and W.A. Houry.[2] Medical Sciences Building, Department of Biochemistry, University of Toronto, 1 King's College Circle, Toronto, ON M5S 1A8, Canada.

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[2]Corresponding author (e-mail: walid.houry@utoronto.ca).

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Hsp90 substrates and cofactors

The molecular chaperone Hsp90 plays a central role in a wide variety of cellular pathways and has been implicated in normal cell development, physiology, disease, and evolutionary processes. In eukaryotes, cytoplasmic Hsp90 is absolutely essential for cell viability under all growth conditions (Borkovich et al. 1989; Cutforth and Rubin 1994). In human cells, there are 2 Hsp90 isoforms expressed in the cytosol (Hsp90α and Hsp90β), which are 78% identical; a third member is present in the lumen of the endoplasmic reticulum (Grp94), and a fourth member is localized in the mitochondria (TRAP1). In the *Saccharomyces cerevisiae* (yeast) cytoplasm, there are 2 virtually identical isoforms of Hsp90: one termed Hsp82, which is heat shock induced, and the other termed Hsc82, which is constitutively expressed.

Hsp90 is an ATP-dependent molecular chaperone that forms a constitutive dimer. Sequence conservation and proteolysis studies of Hsp90s have indicated the presence of at least 3 domains (Fig. 1). The N-terminal domain (residues 1–220 in yeast Hsp82) is the site of ATP binding and hydrolysis. This domain is essential for the ATP-dependent function of the chaperone in vivo and in vitro, and its structure has been solved for the yeast (Prodromou et al. 1997*a*), human (Stebbins et al. 1997), and *Escherichia coli* (Huai et al. 2005) forms. It contains a unique ATP-binding site, which is found in a growing superfamily of proteins termed the Bergerat fold (Dutta and Inouye 2000).

The N-terminal domain is connected through a small, highly charged linker region to a middle domain. No specific function has been attributed to this linker domain. The middle domain (residues 255–599 in yeast Hsp82) has been proposed to be the site of cochaperone and substrate binding as well as a possible site for ATP binding, although this latter result is not well established (Garnier et al. 2002; Soti et al. 2002). The structure of this domain has recently been solved for yeast Hsp82 (Meyer et al. 2003) and *E. coli* Hsp90 (Huai et al. 2005). Finally, the C-terminal domain (residues 600–709 in yeast Hsp82) provides a strong dimerization interface that is essential for Hsp90 function. This domain has a novel fold, whose structure has been solved for *E. coli* Hsp90 (Harris et al. 2004), demonstrating that the dimerization of the domain depends on the formation of a 4-helix bundle.

Hsp90 is distinct from other chaperone systems in 2 main aspects. Unlike the regular chaperone systems, Hsp90 does not act to fold non-native proteins but, rather, binds to substrate proteins that are in a near-native state and, thus, that are at a late stage of folding (Jakob et al. 1995). As an example, Hsp90 binds the apo form of the native glucocorticoid receptor but not the native receptor in the presence of its cognate hormone. Furthermore, Hsp90 seems to be a specialized chaperone, which targets a specific set of client proteins that are mainly involved in signal transduction pathways. Hsp90 substrates include mainly transcription factors and protein kinases (Buchner 1999). Examples of Hsp90-bound transcription factors and polymerases include glucocorticoid receptor, progesterone receptor, estrogen receptor, and p53 mutant. Examples of signaling protein kinases bound by Hsp90 include v-Src, Hck, Wee1, Swe1, Cdc2, Cdk4, and insulin receptor. Other proteins bound by Hsp90 include protease-activated receptor 1, cystic fibrosis transmembrane regulator (CFTR), Fanconi anemia group C protein, and thyroglobulin. This is just a partial list, and a more extensive one can be found in the review by Pratt and Toft (2003) and on the Web site of Dr. Didier Picard (http://www.picard.ch/downloads/Hsp90facts.pdf).

The in vivo and in vitro activity of Hsp90 depends on its association with cochaperones and cofactors that are components of the large Hsp90-multiprotein complexes involved in folding client proteins (Table 1). Some of these cochaperones regulate the Hsp90 ATPase activity, others assist in protein folding, and a third group functions as a scaffold for Hsp90 complexes.

The chaperone Hsp70 and its cochaperone Hsp40 have been found in complex with Hsp90 (Kanelakis et al. 2000). Both Hsp70 and Hsp40 are thought to bind polypeptide chains in an extended conformation and then transfer these proteins to Hsp90. The formation of the complex between Hsp70 and Hsp90 is through the association of both chaperones to an adaptor protein termed Hop/p60 (Sti1 in yeast). Hop is composed of tetratricopeptide repeat (TPR) motifs and DP repeat motifs (Prapapanich et al. 1998; Carrigan et al. 2005) but does not function as a chaperone on its own. A TPR motif consists of 34 amino acids, which form a helix–turn–helix fold. Hop has 8 or 9 TPR motifs forming 3 TPR domains. It is proposed that the N-terminal domain of Hop binds to the C-terminal EEVD motif of Hsp70, and the 2 C-terminal TPR domains bind to the C-terminal EEVD motif of Hsp90 (Scheufler et al. 2000; Young et al. 2001). Hence, Hop forms the scaffold for Hsp90 and Hsp70 complex formation (Prodromou et al. 1999). The Hsp90–Hop–Hsp70 complex is considered to be the minimal Hsp90 core complex. Hsp90-binding TPR domains have also been found in other mammalian cochaperones, including cyclophilin CyP-40, immunophilins FKBP51 and FKBP52, as well as the yeast cyclophilins Cpr6 and Cpr7, and yeast immunophilin Cns1 (Pearl and Prodromou 2002). Another scaffold protein is p50 (yeast Cdc37), which binds, via its C-terminal domain (Silverstein et al. 1998; Roe et al. 2004), to Hsp90 and to many protein kinase client proteins via its N-terminus (Grammatikakis et al. 1999).

Mature Hsp90 complexes with bound substrates have been found to contain the highly conserved protein p23 (Sba1 in yeast), which specifically recognizes the ATP-bound state of Hsp90 and promotes the release of bound client proteins following ATP hydrolysis (Young and Hartl 2000). A cofactor termed Aha1/Hch1 was found to enhance the ATPase activity of Hsp90 (Panaretou et al. 2002; Lotz et al. 2003). A list of established Hsp90 cochaperones–cofactors is given in Table 1.

Hsp90 functional cycle

The Hsp90 functional cycle is not fully understood. The different steps in the cycle are best studied for the case of steroid hormone receptor maturation (Fig. 2A). In the absence of hormone, steroid receptors are inactive and are sequestered by the Hsp90 complex. It has been proposed that the monomeric inactive steroid receptor first binds to Hsp40 in a native-like state with high affinity (Hernandez et al. 2002). Hsp70 is then recruited to this complex through its interaction with Hsp40. The receptor is transferred to Hsp70 in an ATP-dependent reaction. Subsequently, Hop, which can interact

Fig. 1. Hsp90 structure. (A) Schematic representation of the domain boundaries of yeast Hsp82. (B) Structure of the yeast N-terminal (protein data bank (PDB) code 1AM1), yeast middle (PDB code 1HK7), and *Escherichia coli* C-terminal (PDB code 1SF8) Hsp90 domains. The *E. coli* Hsp90 C-terminal domain forms a dimer. Structures were drawn using PyMOL (http://pymol.sourceforge.net).

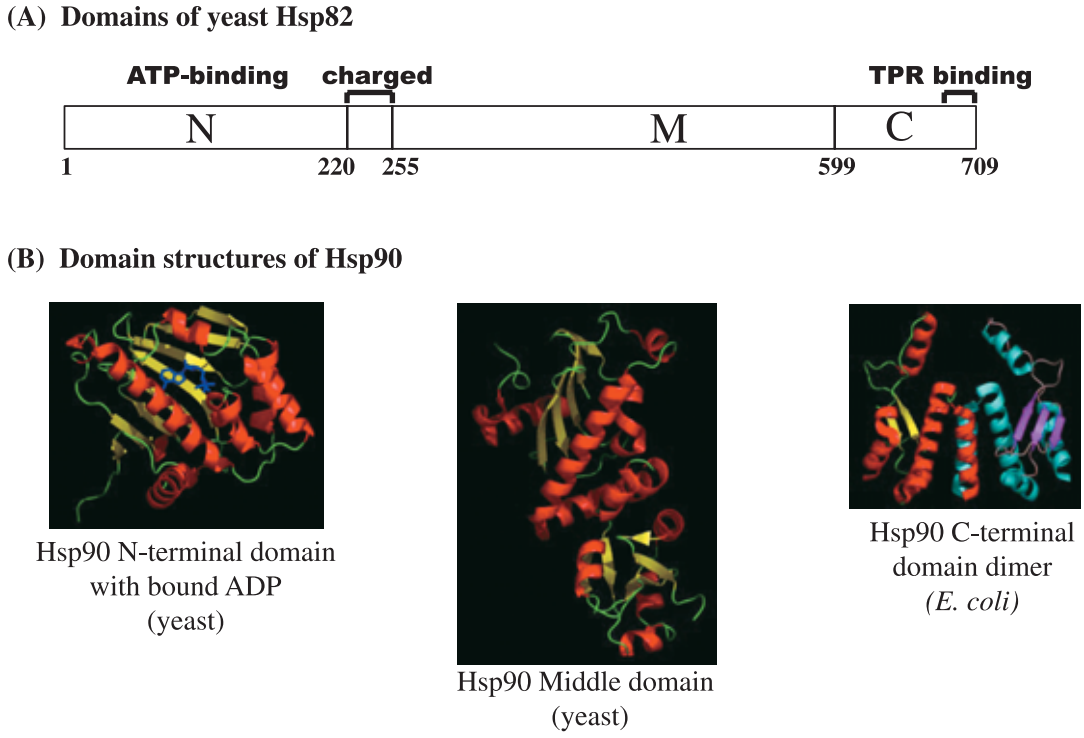


Table 1. Hsp90 cofactors and cochaperones.

General name	Name in yeast	Name in higher eukaryotes	Function
Hsp70	Ssa1	Hsp70	Chaperone
Hsp40	Ydj1	Hsp40	Chaperone
p23	Sba1	p23	Recognizes the ATP-bound state of Hsp90
Hop	Sti1	Hop/p60	Scaffold protein for Hsp90 and Hsp70
Aha1	Aha1	hAha1	Enhances ATPase of Hsp90
Cyclophilins	Cpr6, Cpr7	Cyp-40	Possible prolyl isomerase
Immunophilins	CNS1	FKBP51, FKBP52	Possible prolyl isomerase
Cdc37	Cdc37	p50	Kinase specific cofactor
CHIP	Not known	CHIP	Links Hsp90 to the degradation machinery
Tah1	Tah1	Not known / SGTβ?	Links Hsp90 to Rvb1/2
Pih1	Pih1	FLJ20643	Links Hsp90 to Rvb1/2

with both Hsp70 and Hsp90, promotes the binding of Hsp90 to the Hsp70 complex to form the intermediate complex that contains substrate Hsp70–Hop–Hsp90 (Chen and Smith 1998; Morishima et al. 2000). The intermediate complex is then converted to the mature complex containing substrate, Hsp90, and p23. The mechanism of this conversion is not known but involves the binding of Hsp90 to ATP, which is stabilized by p23. In this complex, the hormone-binding domain of the steroid receptor is maintained in a conformation capable of binding hormone. Once the folded monomeric receptor has been released from the chaperone, it either binds hormone, resulting in dimerization and activation, or it remains unstable and is bound again by the chaperone machinery.

The Hsp90 dimer, which is formed through the association of the C-terminal domains, undergoes a large conformational change during its ATP-dependent functional cycle. Two extreme conformational states for the Hsp90 dimer have been observed (Maruya et al. 1999; Prodromou et al. 2000) (Fig. 2B): a "tense" state, in which ATP binding to Hsp90 results in the association of the N-terminal domains in the dimer causing the formation of a closed toroidal structure, and a "relaxed" state, in which the N-terminal domains are dissociated. It has been suggested that the toroidal ATP-bound structure of Hsp90 might function as a clamp to hold client proteins that are then released upon ATP hydrolysis and opening of the clamp (Pearl and Prodromou 2002).

Hsp90 as an antitumor drug target

Several fungal antibiotics belonging to the benzoquinone

Fig. 2. Hsp90 mechanism of function. (A) The maturation of steroid hormone receptor as mediated by Hsp90. (B) The proposed nucleotide-dependent conformational changes in Hsp90.

(A) Hsp90 functional cycle

Steroid Hormone Receptor (SHR) + Hsp40 → Hsp40-SHR

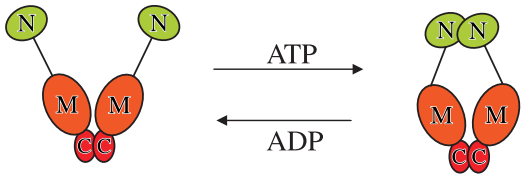
Hsp40-SHR + Hsp70 → Hsp40-SHR-Hsp70

Hsp40-SHR-Hsp70 + Hop-Hsp90 → Hsp40-SHR-Hsp70-Hop-Hsp90 *intermediate complex*

Hsp40-SHR-Hsp70-Hop-Hsp90 + p23 → Hop + Hsp70 + Hsp40-SHR-Hsp90-p23 *mature complex*

Hsp40-SHR-Hsp90-p23 —hormone→ Hsp40 + SHR + Hsp90 + mature SHR (bound to hormone)

(B) ATP-induced N-terminal dimerization of Hsp90



ansamycin family, such as geldanamycin, herbimycin A, and macbecin, have been found to possess antitumor activity through promotion of the degradation of several proto-oncogenic protein kinases, such as v-Src or Raf-1 (Whitesell et al. 1994; Schneider et al. 1996). These antitumor drugs do not bind directly to the kinases but, rather, to Hsp90. It has been shown that geldanamycin directly binds to the ATP-binding pocket in the N-terminal domain of Hsp90 (Prodromou et al. 1997*b*; Stebbins et al. 1997) and, hence, blocks the binding of nucleotides to Hsp90. Analysis of the effects of geldanamycin on steroid receptor activation indicates that the antibiotic blocks the chaperone cycle at the intermediate complex (Fig. 2A), preventing the release of the receptor from Hsp90 and, eventually, resulting in its degradation.

Since molecular chaperones are overexpressed in a wide variety of cancer cells and in virally transformed cells, inhibiting the function of these chaperones is essential to controlling cancer cells, as this would affect the activity of signaling proteins. The availability of drugs that can specifically target Hsp90 and inhibit its function, resulting in the depletion of client proteins, has made Hsp90 a novel and exciting target for cancer therapy (Neckers 2002). A geldanamycin derivative, termed 17-(allylamino)-17-demethoxygeldanamycin (17-AAG), is now progressing to phase II clinical trials (Dunn 2002; Maloney and Workman 2002; Workman 2002). In an elegant study, Kamal et al. (2003) demonstrated that 17-AAG preferentially affects Hsp90 in cancer cells because Hsp90 in these cells is present entirely in multichaperone complexes with high ATPase activity and high affinity for 17-AAG, whereas Hsp90 in normal cells is mainly in a latent, uncomplexed state with low affinity for 17-AAG.

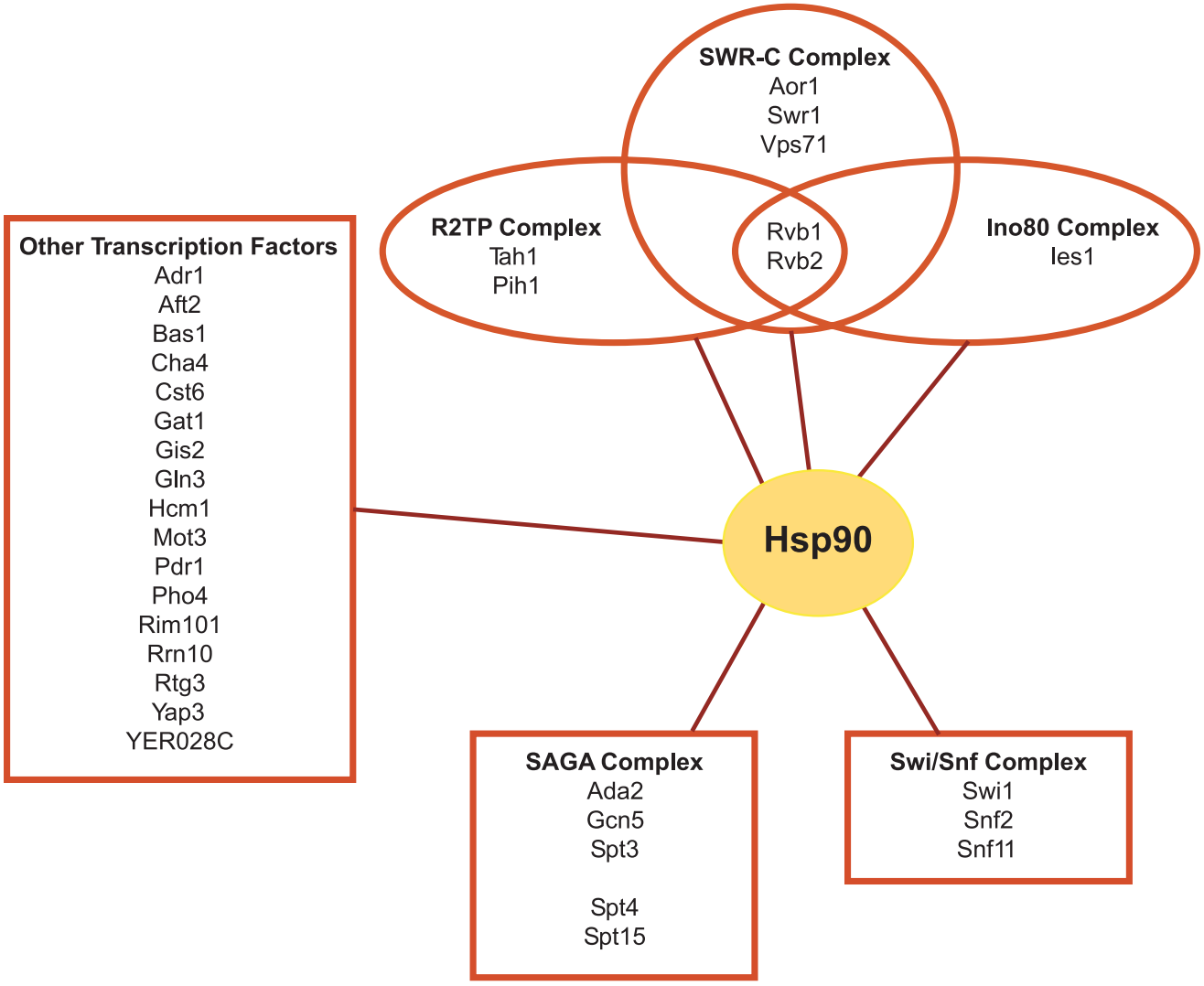
The Hsp90 chaperone network

We started a research program aimed at investigating the global role of the Hsp90 system inside the cell. We carried out comprehensive proteomic screens using yeast to identify novel interacting partners, substrates, and cofactors of yeast Hsp90. The effort was part of a large collaboration among several groups at the University of Toronto (Zhao et al. 2005).

Our proteomics efforts were based on a combination of 4 complementary experimental strategies. (*i*) We carried out systematic genome-wide screens for protein–protein interactions mediated by Hsp90 using a 2-hybrid technique based on ordered strain arrays (Uetz et al. 2000). (*ii*) We used protein mass spectrometry to identify proteins that copurify with Hsp90 in tandem affinity purification (TAP) procedures in which Hsp90 or yeast open reading frames (ORFs) were chromosomally TAP-tagged (Puig et al. 2001); the tag was used to pull down Hsp90-containing complexes, and polypeptides in those complexes were identified by mass spectrometry. The tag consists of tandem copies of the IgG-binding units from *Staphylococcus aureus* protein A, followed by the recognition site for the highly specific tobacco etch virus protease, which is then followed by the sequence encoding a recombinant calmodulin-binding peptide. The TAP-tag confers sufficient affinity for 2 distinct ligand-based affinity resins such that recovery of proteins present at low abundance in a complex mixture can readily be achieved. (*iii*) We screened for synthetic lethal interactions between a temperature-sensitive mutant allele of Hsp90 and members of a panel of ~4700 single yeast gene deletion strains using synthetic genetic array technology (Tong et al. 2001). (*iv*) We screened the deletion mutant strains for differential hypersensitivity to the Hsp90 inhibitor geldanamycin in liquid culture using a microarray-based readout (Giaever et al. 2002). Hence, our efforts were aimed at identifying physical as well as genetic interactors of yeast Hsp90.

These efforts have resulted in the identification of about 600 potential Hsp90 cofactors and substrates out of the ~6000 yeast ORFs. Several candidates have been further screened using biochemical assays. Two of these proteins,

Fig. 3. The effect of Hsp90 on gene regulation. Listed are transcription factors and proteins that are components of chromatin remodeling–modification complexes that were found to interact physically or genetically with Hsp90 in our large-scale screens (Zhao et al. 2005). However, Rvb2 was not found to interact with Hsp90 but was added to the figure to emphasize that Rvb1 and Rvb2 are common to the R2TP, SWR-C, and Ino80 complexes.



termed Tah1 (systematic name YCR060w, 111 residues) and Pih1 (systematic name YHR034c, 344 residues), showed very interesting biochemical characteristics and suggested a novel mechanism by which Hsp90 might exert its influence on protein homeostasis in the cell. Tah1 and Pih1 were found to interact with Hsp90 and were related to uncharacterized human proteins (Table 1). Tah1 is a small TPR domain containing protein. Pih1 is highly conserved but has no known domains or motifs. The 2 proteins physically interact with each other, and they form specific complexes with the DNA helicases Rvb1 and Rvb2. These helicases are essential components of the chromatin remodeling machinery (Shen et al. 2000; Krogan et al. 2003; Mizuguchi et al. 2004; Kobor et al. 2004). We postulate that Tah1 and Pih1 target Hsp90 to Rvb1 and Rvb2, which would allow the chaperone to indirectly affect gene regulation under different conditions (Fig. 3). In this regard, we also uncovered interactions between Hsp90 and proteins (or genes) of the Spt-Ada-Gcn5-Acetyltransferase (SAGA) and Swi/Snf complexes, involved in chromatin remodeling, as well as a large number of transcription factors (Fig. 3). Consequently, we are proposing a novel role of Hsp90 in gene regulation mainly by its regulation of the folding, activity, maturation, or complex formation of proteins involved in chromatin remodeling. There are 2 lines of evidence in the literature that suggest such a novel role: one relates to nuclear-associated activities reported for the chaperone, and the other relates to the ability of the chaperone to function as a capacitor for morphological development in a genetic or epigenetic manner.

Nuclear-associated activities of Hsp90

Freeman and Yamamoto (2002) recently demonstrated that Hsp90 and its cochaperone p23 promote the disassembly of receptor-mediated transcriptional regulatory complexes. Using the chromatin immunoprecipitation assay, they found that p23 and Hsp90 localized to the glucocorticoid response elements in a hormone-dependent manner, whereas Hsp70

was not detected. This indicates that p23 and Hsp90 are selectively recruited to functional glucocorticoid receptor - containing regulatory complexes in vivo. Furthermore, Hsp90 and p23 have been shown to physically interact with telomerase and are required for the function of telomerase complex (Holt et al. 1999). The reduction of Hsp90 expression level affects the telomere length (Grandin and Charbonneau 2001). These findings suggest that Hsp90 and its cochaperone are directly involved in the formation and maintenance of a certain state of the chromatin and possibly are necessary for the stability of the chromatin.

Hsp90 has also been found to play an essential role in chromosome segregation during mitosis. Hsp90 and Sgt1 are required for the Ctf13/Skp1 complex formation (Kitagawa et al. 1999; Stemmann et al. 2002), which is the first step in nucleating the kinetochore in yeast. It was found that Hsp82 has a synthetic lethal interaction with Skp1. With decreased levels of Hsp90 or inhibition of the Hsp90 function by addition of geldanamycin, the formation of Ctf13/Skp1 complex is prevented (Stemmann et al. 2002). Biochemical and genetic studies suggest that Skp1p and Sgt1p modify Ctf13p through protein phosphorylation and the action of Hsp90 (Stemmann et al. 2002; Rodrigo-Brenni et al. 2004; Lingelbach and Kaplan 2004). The association of Sgt1 with Hsp90 was also identified in our TAP-tag pulldown screens (Zhao et al. 2005), suggesting that Sgt1 may function as a cochaperone of Hsp90, specifically for the formation of kinetochore complex. Sgt1 has an N-terminal TPR-like domain and a cysteine- and histidine-rich domain (CHORD); both domains are possible Hsp90 interaction domains.

The nuclear-associated activities of Hsp90 suggest that at least a certain population of the chaperone functions within the nuclear environment. Indeed, there are several indications that some of the Hsp90 resides in the nucleus of eukaryotic cells. In plants, Hsp90 was found to be distributed between the cytoplasm and nucleus. Nuclear Hsp90 was mostly found in the interchromatin region (Segui-Simarro et al. 2003). Furthermore, upon temperature upshift (to 32 °C), Hsp90 was induced and translocated from the cytoplasm to the nucleus. In mammalian cells, slightly increased nuclear accumulation of Hsp90 was found upon hypoxic shock (Katschinski et al. 2002).

The main localization of Hsp90 is in the cytoplasm. This could be partially due to the existence of the cytoplasmic localization signal in the Hsp90 protein (Passinen et al. 2001). However, subfragments of Hsp90 might be prone to nuclear localization. Hsp90 is very abundant in the cell, therefore its synthesis and turnover must be highly dynamic. The chance of Hsp90 subfragments existing in vivo is very high. Actually, Piper and colleagues reported that Hsp90 from both *Candida albicans* and *S. cerevisiae* generate many subfragments in *S. cerevisiae* with predominantly 72–76 kDa and 47 kDa molecular mass (Panaretou et al. 1999), but the function of these subfragments remains to be clarified. The mechanism that generates the subfragments is unknown, but since we also noticed that subfragments of similar molecular mass were generated when isolating the N-terminally TAP-tagged Hsp90, it seems that this might be a common phenomenon. The subfragments of Hsp90 in vivo may play some yet unknown physiological functions. In this regard, the C-terminal fragment of Hsp90, especially the hydrophobic region (residues 657–720 in human Hsp90α), has been shown to bind to client proteins and to be sufficient to prevent the aggregation of the model substrate citrate synthase (Yamada et al. 2003). Similarly, the C-terminal fragment (residues 494–782) of the human GRP94 protects the catalytic subunit of protein kinase CK2 against thermal aggregation (Roher et al. 2001). This suggests that the fragments of Hsp90 generated in vivo might have potential chaperone activity concomitant with their nuclear localization.

Hsp90 as a capacitor for morphological development

Rutherford and Lindquist (1998) first showed that the reduction of Hsp90 levels reveals concealed phenotypic variations in *Drosophila*, suggesting that Hsp90 buffers such variations. Subsequently, Lindquist and coworkers showed that *Arabidopsis* isolates can have dramatic phenotypic variation when Hsp90 function is challenged, indicating that the buffering of genetic variations is conserved across plant and insect kingdoms (Queitsch et al. 2002). The buffering ability of Hsp90 reported by Lindquist and coworkers was explained by the ability of the chaperone to directly mask the phenotypic effects of mutant polypeptides by allowing their correct folding, or indirectly by regulating signal transduction to buffer the phenotypes (Rutherford 2003). Consequently, the authors explained their observation as being the result of previously hidden genetic variations that become exposed if Hsp90 function is compromised.

Sollars et al. (2003) provided evidence that Hsp90 might also work by an epigenetic mechanism to buffer some evolutionary mutations. Epigenetic refers to mitotically and (or) meiotically heritable changes in gene expression that do not involve a change in DNA sequence. Epigenetic variation does not follow the rules of Mendelian inheritance, is often the result of changed gene expression, and may be reversible. Sollars et al. found that, in *Drosophila*, Hsp90 affects development in an epigenetic manner by altering the chromatin in the eye imaginal disc. They propose that reduction of Hsp90 levels sensitizes the target chromatin for activation or repression. It remains controversial whether Hsp90 exerts its influence through genetic or epigenetic mechanisms (Sangster et al. 2003), but the many interactions uncovered by our global proteomic effort (Zhao et al. 2005) between Hsp90 and chromatin remodeling complexes might favor an epigenetic effect of the chaperone (Fig. 3).

Conclusion

In our recent proteomics work (Zhao et al. 2005), we have expanded the set of Hsp90 client proteins, and we have also identified new cofactors of the chaperone. We found that around 10% of the yeast proteome physically or functionally interacts with the chaperone. The global role of Hsp90 was evident from its effect on a wide array of cellular processes. More importantly, a possible role of Hsp90 in the regulation of gene expression is strongly suggested, since we found the chaperone to interact with several chromatin remodeling complexes, including SAGA, Swi/Snf, Ino80, and SWR-C, as well as many transcription factors (Fig. 3). In the case of Ino80 and SWR-C, we have identified novel cofactors of the

chaperone that link Hsp90 to essential DNA helicases that are part of these complexes. Efforts in our laboratory are currently focused on determining, at the molecular level, how Hsp90 exerts its influence on chromatin through these novel cofactors.

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