Chapter 5
The Interaction Network of the Hsp90 Molecular Chaperone

Kamran Rizzolo, Philip Wong, Elisabeth R. M. Tillier and Walid A. Houry

Abstract Heat shock protein 90 (Hsp 90) is a highly abundant and critical molecular chaperone that plays key roles in cellular quality control systems. The Hsp90 mechanism of function has been the subject of extensive investigation by many groups using traditional biochemical approaches as well as high-throughput methods, however, the Hsp90 functional cycle still remains enigmatic. A complicating factor in understanding Hsp90 function is the presence of many cofactors and co-chaperones that assist in Hsp90 chaperoning activity. The widely used model organism, *Saccharomyces cerevisiae* (budding yeast), contains two highly conserved members of the Hsp90 family, Hsp82 and Hsc82, and has been used as a model organism for mapping Hsp90 interactors. High-throughput proteomic studies on the yeast Hsp90 provided a wealth of information from a global perspective. More recently, such studies in mammalian cells have led to a better understanding of Hsp90 function. Here, we discuss the Hsp90 functions and highlight the most recent efforts leading to the construction of the Hsp90 interaction networks.

1 The Hsp90 Chaperone

The heat shock protein 90 (Hsp 90) is a member of a widespread family of molecular chaperones found in both eukaryotes and prokaryotes. Hsp90s are highly abundant within the cell accounting for about 1–2 % of total protein [1, 2]. Bacterial Hsp90s are typically non-essential, however, a functional Hsp90 is required for cell viability in eukaryotes [3, 4]. In mammalian cells, Hsp90 is predominantly localized to the cytoplasm and the nucleus but is also present in several organelles such as the mitochondria and endoplasmic reticulum [5].

Hsp90 functions as a homo-dimeric protein, with each monomer consisting of three domains: the N-terminal domain (N-domain) involved in adenosine triphosphate (ATP) binding and hydrolysis, a middle domain involved in substrate binding

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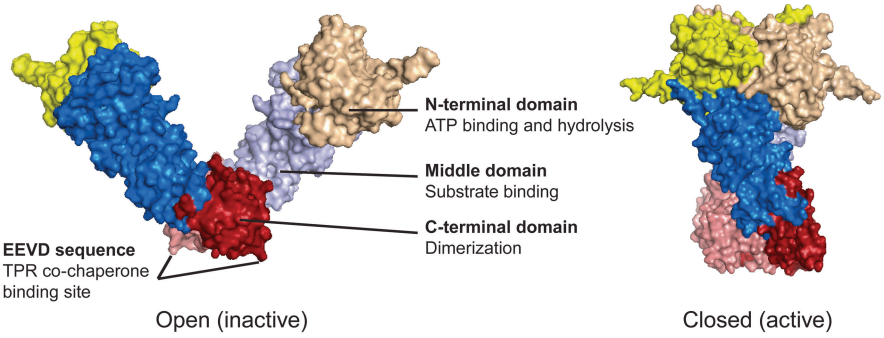

Fig. 5.1 Heat shock protein 90 (Hsp90) structure and conformational states. The Hsp90 homodimer with three domains in each monomer is shown: The N-terminal domain (N-domain in *yellow* and wheat) is the nucleotide binding domain (NBD) and is primarily responsible for adenosine triphosphate (ATP) binding and hydrolysis, the middle-domain (M-domain in *blue* and *sky blue*) has been implicated in client binding while the C-terminal domain (C-domain in *red* and salmon) is responsible for dimerization and contains the tetratricopeptide repeat (TPR) co-chaperone binding motif sequence EEVD at the very C-terminus. The structure on the *left* shows the inactive open form of *Escherichia coli* Hsp90 (PDB entry 2IOQ) [93]. The structure on the *right* shows the closed active form of *S. cerevisiae* Hsp90 (PDB entry 2CG9) [43].

(M-domain), and a C-terminal domain necessary for dimerization (Fig. 5.1) [6, 7]. Hsp90 has hundreds of substrate proteins, which are referred to as clients, belonging to different protein families but have no obvious sequence, structural, or functional similarities [8, 9]. It has been shown that yeast Hsp90 interacts genetically or physically with ~10% of the yeast proteome [8]. Also, it is speculated that Hsp90 in mammalian cells may assist in the folding and maturation of up to 10% of cytosolic proteins at some stage in their life cycle [2]. The majority of Hsp90 client proteins play essential roles in many biological processes and, therefore, Hsp90 is a master regulator of very diverse cellular activities including: signal transduction, vesicle transport, cell cycle regulation, telomere maintenance, transcription regulation, steroid signaling, immune response, viral infections, and cancer development [10, 11]. Hsp90 is currently considered as a validated anticancer drug target [12].

One key difference between Hsp90 and other chaperones is that Hsp90 can interact with a great number of co-chaperones and, in turn, these associations are critical for its activity [9]. The co-chaperones regulate Hsp90 ATPase activity, influence the chaperone's affinity for client proteins, or cause the chaperone to be targeted to a particular cellular pathway. Traditional biochemical and genetic approaches to characterize Hsp90 clients, co-chaperones, and other cofactors have been challenging primarily due to the low affinity of the chaperone-substrate interactions and the pleiotropic functions of this chaperone [1, 2, 13]. Biochemical and genetic studies suggest that Hsp90 is not a chaperone that interacts with unfolded or newly translated proteins, rather it seems to act on proteins that are significantly folded. Hsp90 most likely plays a role in remodeling protein conformation or in protein stabilization [6, 14].

The model organism *Saccharomyces cerevisiae* has served as an ideal system in providing a global cellular view of Hsp90 functions along with the discovery of novel interactors. There are two isoforms of Hsp90 in yeast: one that is constitutively expressed (Hsc82) and another one that is induced under heat shock conditions (Hsp82) [15]. The two proteins are 97% identical at the protein sequence level [3]. In this review, we provide an overview of the Hsp90 functions and discuss studies involving Hsp90 chaperone network characterization including methods used and possible future directions.

2 Hsp90 Co-chaperones

Given the breadth of Hsp90 involvement in various cellular pathways, an efficient control mechanism is needed to ensure proper function and avoid deleterious effects that may occur when the activity of this abundant chaperone is not regulated. The regulation of Hsp90 function is performed by numerous co-chaperones that have co-evolved with Hsp90 [16]. The repertoire of co-chaperones varies greatly between eukaryotes and may well be dependent on the presence of specific client proteins [9, 17]. More than 20 co-chaperones are known to be involved in affecting the ATP-dependent functional cycle of Hsp90 and the recruitment of client proteins (Fig. 5.2). By taking into account the effect on Hsp90 and its clients' maturation, co-chaperones can be divided into three groups: client recruiters, Hsp90 remodelers, and late-acting co-chaperones. These groups have overlapping members [9].

Many co-chaperones of Hsp90 contain the tetratricopeptide repeat (TPR) domains that bind the C-terminal Glu-Glu-Val-Asp (EEVD) sequence present in eukaryotic cytoplasmic Hsp90s [9]. These TPR domains are typically composed of tandem helical repeats of seven antiparallel α-helices that form a cleft capable of binding the EEVD C-terminal residues of Hsp90 and also Hsp70 [18]. Some important TPR domain-containing co-chaperones of Hsp90 are listed in Table 5.1. Heat shock organizing protein (Hop; yeast Sti1) has three TPR domains and forms a ternary complex with Hsp90 and Hsp70 [19, 20]. Protein phosphatase PP5 (yeast Ppt1) is involved in the maturation of the glucocorticoid receptor [21, 22]. Yeast Tah1 [8, 23] and mammalian RPAP3 [24] form a ternary complex with Hsp90 and another protein termed Pih1 involved in the assembly of box C/D small nucleolar ribonucleoprotein complexes (snoRNPs). Myosin-folding factor Unc45 (yeast She4) is involved in myosin fiber assembly [25, 26]. Members of the peptidylprolyl isomerase (PPIase) family Cyp40 (yeast Cpr6/Cpr7) [17, 27] are involved in the maturation of certain clients. Tom70 is involved in mitochondrial import [28]. Sgt1 is a subunit of the core kinetochore and the Skp1-Cul1-F-box (SCF) ubiquitin ligase complex [29]. However, it should be noted that unlike other TPR-domain-containing co-chaperones of Hsp90, Sgt1 binds Hsp90 through its p23-like cysteine and histidine-rich domain (CHORD) and Sgt1 (CS) domain and not through its TPR domain [30].

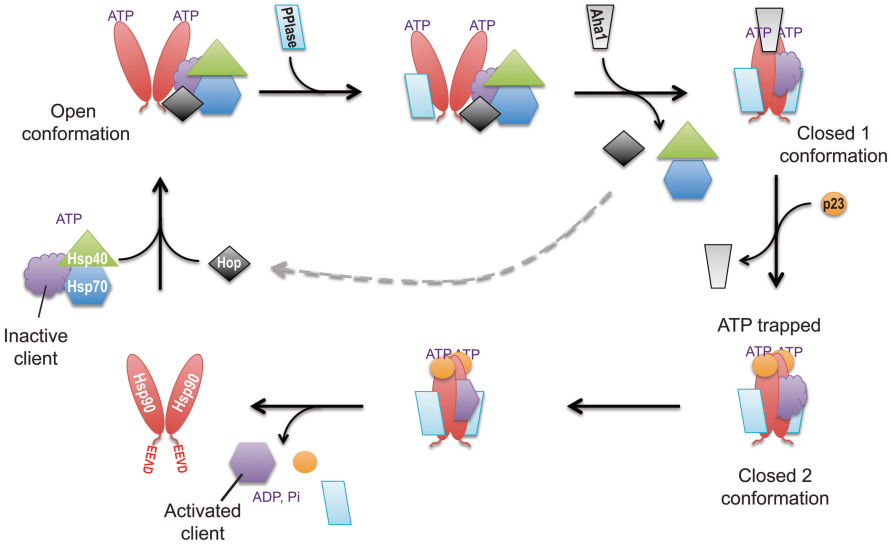

Fig. 5.2 Heat shock protein 90 (Hsp90) cycle and client maturation. Heat shock organizing protein (Hop) binds to the open state of Hsp90 together with the Hsp70-Hsp40-client protein complex. The adenosine triphosphate (ATP) hydrolysis-inhibited state may allow the binding of peptidyl-prolyl isomerase (PPIase) producing an asymmetric Hsp90 protein complex. One or two PPIases might bind the Hsp90 dimer. ATP binding to the complex induces a conformational change causing the transfer of the client from Hsp70 to Hsp90. Co-chaperone Aha1 binds Hsp90 and weakens the Hop-Hsp90 interaction causing the release of Hop from the chaperone. As a result, the Hsp70-Hsp40 complex is also released. Aha1 induces a conformational change in Hsp90 producing the closed state (closed 1). The resulting Hsp90-client complex is stabilized by the binding of the p23 co-chaperone and leads to the release of Aha1 (closed 2). Subsequently, ATP hydrolysis generates a conformational change that releases the mature client protein, the PPIase, and p23. The EEVD sequence at the C-terminus of Hsp90 is shown.

Other less-characterized TPR-domain-containing co-chaperones of Hsp90 (Table 5.1) include the essential yeast protein Cns1 whose activity is poorly understood but might overlap with that of Cpr7 [31]. Ttc4 has been postulated to link Hsp90 chaperone activity with deoxyribonucleic acid (DNA) replication [32]. The J-domain containing protein TPR2 is proposed to play a role in the Hsp90-dependent chaperoning of the progesterone receptor [33, 34].

There are three main co-chaperones of Hsp90 that do not contain a TPR domain (Table 5.1). Aha1 enhances the Hsp90 ATPase activity and acts to generally enhance Hsp90's chaperoning function [35–37]. Aha1 forms an intricate complex with Hsp90: the Aha1 N-terminal domain binds to Hsp90's middle domain while the C-terminal domain of Aha1 interacts with the N-terminal domain of the chaperone [36, 38–40]. The close paralog of Aha1, Hch1, also enhances Hsp90 ATPase activity. However, despite having more than 50% amino acid similarity with Aha1, Hch1 has been shown to regulate Hsp90 function in a way distinct from that of Aha1 and independent from Hch1's effect on Hsp90 ATPase activity [41]. p23 is an abundant co-chaperone of Hsp90. It forms co-complexes with the chaperone

Table 5.1 Hsp90 co-chaperones

Co-chaperones		Function
Yeast	Mammals	
TPR co-chaperones		
Sti1	Hop	Scaffold protein for Hsp90/Hsp70, involved in maturation of client proteins, inhibits Hsp90 ATPase
Ppt	PP5	Phosphatase
Tah1	RPAP3	Forms complex with Pih1 and Hsp90
She4	Unc45	Involved in Myosin fiber assembly
Cpr6/Cpr7	Cyp40	Peptidy-prolyl-isomerase, involved in maturation of client proteins
Tom70	Tom70	Involved in Mitochondrial protein import
Sgt1	Sgt1	Involved in kinetochore assembly
Cns1		Not well characterized, essential in yeast
	Ttc4	Acts as a link between Hsp90 and DNA replication
	TPR2	Involved in the maturation of the progesterone receptor
Non-TPR co-chaperones		
Aha1/Hch1	Aha1	Stimulates ATPase activity of Hsp90 and modulates the conformational changes of the chaperone
Sba1	p23	Inhibits Hsp90 ATPase activity, has independent chaperone activity
Cdc37	Cdc37	Inhibits Hsp90 ATPase activity, targets Hsp90 to kinases

TPR Tetratricopeptide, *ATP* Adenosine triphosphate, *Hop* Heat shock organizing protein, *Hsp* Heat shock protein, *DNA* Deoxyribonucleic acid

and client proteins, however, it might also function independently [42]. p23 binds the N-terminal domain of Hsp90 and stabilizes the closed ATP-bound state of the chaperone (Fig. 5.2) [43]. Finally, the cell division cycle 37 protein (Cdc37) acts as the co-chaperone of Hsp90 for kinase client proteins [44]. Cdc37 binds both the N-terminal and middle domains of the chaperone and restricts Hsp90 conformational mobility [45–47]. As a result, Cdc37 stabilizes the client-loading phase of the chaperone cycle prior to the dimerization of the Hsp90 N-terminal domains and inhibits the chaperone's ATPase [9] (Fig. 5.2).

The Hsp90 functional cycle is not fully elucidated and might not be strictly regulated by ATP binding and hydrolysis [48, 49]. It is well established that the Hsp90 cycle also involves the Hsp70 and Hsp40 chaperones (Fig. 5.2). Hsp70 has an N-terminal nucleotide-binding domain (NBD) followed by a substrate-binding domain (SBD) and a C-terminal region. Hsp40 has a J-domain that binds to the Hsp70 NBD and enhances the ATPase activity of Hsp70 [50]. In a simplistic model of the Hsp90 cycle (Fig. 5.2), substrates initially bound by Hsp70-Hsp40 are transferred to Hsp90 through the bridging action of Hop that interacts with both Hsp90 and Hsp70. There is a minimal complex consisting of Hsp40, Hsp70, and Hsp90 and including the co-chaperone Hop that has been shown to form with clients such as the hormone receptors [51, 52]. Subsequently, Aha1 and then p23 stabilize the closed ATP-bound and client-loaded state of Hsp90 resulting in the release of Hsp70-Hsp40. Upon ATP hydrolysis by Hsp90, the client protein is released from the chaperone in a mature

folded state (Fig. 5.2). Certain PPIases also act during this cycle on the Hsp90-containing complexes (Fig. 5.2). It should be noted that Hsp90 has very weak ATPase activity that is regulated by the different cofactors.

3 Coevolution of Hsp90 and Its Co-chaperones

Coevolution of protein-protein interactions have been used to study the interaction of Hsp90 with its co-chaperones (Table 5.1). Coevolution analysis highlights important genetic factors responsible for the robustness of biological systems. Furthermore, NxN sequence comparisons of homologous proteins produce distance matrices that allow the construction of phylogenetic trees [53]. Alignment of different sequences from many homologs enables statistical inference of coevolution between residue positions within the same protein family or between protein families. When homologous residues are aligned, mutations at the homologous site that coincide with mutations at other homologous positions tend to be easier to detect.

Travers and Fares [54] investigated the presence of coevolving amino acid residues between Hsp70 and Hop, as well as, between Hsp90 and Hop. They explored 25 organisms for Hop-Hsp70 and 27 organisms for Hop-Hsp90 inter-protein coevolution. They then used the coevolution analysis using protein sequences (CAPS) method [55] to identify coevolving groups. Such a computational method can suggest related constraints over large evolutionary distances between proteins and is an alternate way to determine biological associations between them. For Hsp70, the authors found a total of 30 amino acid residues coevolving with Hop. The majority of these residues lie within the NBD or the C-terminal region (50% of the total) with only four residues in the SBD. For Hsp90, 51 amino acid residues where observed to coevolve with Hop. The majority of the residues were located within the N-terminal and middle domains. They also found great conservation and overlap between the sites in Hop and the various domains in Hsp90 suggesting inter-domain communication as essential for Hsp90 function.

Furthermore, the Hsp90-Hsp70-Hop system was found to involve complex interactions at the intra-molecular level: A residue change in one domain was found to have effects on domains from other proteins even if they do not directly interact with each other. For example, the first TPR domain of Hop, which binds to Hsp70, was found to have four residues that coevolve with Hsp90, two of which also coevolve with Hsp70. These results demonstrate coevolution at the systems level in the Hsp90 network and further highlight the functional dependence between its components.

4 Posttranslational Modifications of Hsp90

Hsp90 activity is also regulated by posttranslational modifications such as phosphorylation, acetylation, nitrosylation, and SUMOylation (conjugation with small ubiquitin-like modifier) [9, 17, 56]. This confers an additional layer of modulation

of Hsp90 activity by the extra- and intracellular environment in order to guarantee a fast and efficient response to specific cellular requirements [17, 57]. These modifications greatly affect Hsp90 ATPase activity, conformational changes, dimerization, co-chaperone binding, and, as a result, impact client maturation.

Hsp90 is a phosphoprotein whose levels of phosphorylation vary greatly depending on its cellular environment. CK2 is a serine-threonine acidophilic kinase whose activity is highly dependent on Hsp90 function. In yeast, residue T22 in the N-domain of Hsp90 is phosphorylated by CK2, which then modulates the ATPase activity of the chaperone. Mutations of this residue have been shown to affect Hsp90 chaperoning of kinase and non-kinase clients [58, 59]. These mutants significantly affected the interaction of Hsp90 with its co-chaperone Aha1. Interestingly, overexpression of Aha1 can compensate for the various chaperoning defects caused by mutating T22 in Hsp90 [58].

Yeast Swe1 (also known as Wee1) tyrosine kinase is an Hsp90 client that regulates G2/M cell cycle transition [57]. Phosphorylation of Hsp90 in S-phase by this enzyme causes the chaperone to be translocated from the nucleus to the cytoplasm where it is tagged for degradation by proteasomes as a way to eliminate this particular population of Hsp90 in the cell [60]. In addition, deletion of the *SWE1* gene causes increased sensitivity towards Hsp90 inhibiting drugs.

Acetylation of Hsp90 was also shown to regulate Hsp90 functions in client maturation and co-chaperone binding [61]. Nitrosylation (the attachment of nitric oxide, NO, to the thiol side chain of cysteine) of Hsp90 has been found to occur at the C-terminal domain residue C597 of Hsp90α in endothelial cells [62, 63]. This modification inhibits Hsp90 ATPase and chaperone activities. Hsp90 also undergoes asymmetric SUMOylation at a conserved lysine in the N-terminal domain (K178 in yeast Hsp82, K191 in human Hsp90). This modification facilitates the interaction of Aha1 with the chaperone, as well as, the binding of Hsp90 inhibitors [56].

In summary, these studies highlight the importance of various posttranslational modifications of Hsp90 on the ATPase activity, maturation of clients, co-chaperone interactions, conformational changes, and Hsp90 stability.

5 Examples of Hsp90 Clients

In order to understand the diversity in functions of the Hsp90 system, it is crucial to identify the chaperone's client proteins, and more importantly discover the link between them. Despite the increasing list of clients, very little is actually known about client recognition and binding sites. These clients are frequently intrinsically unstable proteins and bind to Hsp90 in a partially unfolded (or folded) state [9]. They are very diverse in structure and function suggesting that Hsp90 interacts with them using a general principal of recognition, probably modulated by the co-chaperones, as opposed to binding specifically to defined motifs.

The instability of Hsp90 clients makes Hsp90-client interaction and structural studies very difficult. Many in vivo and in vitro studies have approached this challenge by combining multiple biochemical and cell biological methods. For example,

Table 5.2 Examples of Hsp90 clients in yeast

Client protein	Description	Client class	Co-chaperones involved	Reference
Ste11	MEK kinase involved in pheromone response	Protein kinase	Cdc37	[66]
Gcn2	Stimulates translation of the yeast transcription factor Gcn4 upon amino acid starvation.	Transcription factor	Cdc37, Sti1, Sba1	[68]
Swe1	Protein kinase that regulates the G2/M transition	Protein kinase	Sba1, Cpr6/Cpr7	[71]
Slt2	Serine/threonine MAP kinase	Protein kinase		[72]
Hap1	Heme-responsive zinc finger transcription factor	Transcription factor	Hsp40 (Ydj1), Hsp70 (Ssa1–4)	[74]
Mal63	MAL activator, a DNA-binding transcription activator	Transcription factor	Cpr7	[76]

DNA Deoxyribonucleic acid, *Hsp* Heat shock protein

Genest et al. [64] developed a screen to identify *E. coli* mutants defective in Hsp90 function. They found that mutations in the M and C domains of Hsp90 affect chaperone activity and client binding. Interestingly, after making the same mutations in yeast Hsp90 (Hsp82), the authors found that mutant Hsp82 was less affected in client-binding capacity. This suggested that Hsp90 client-binding capabilities have diverged through evolution. Table 5.2 lists a few examples of Hsp90 clients that have been studied in some detail.

The first yeast Hsp90 client identified was Ste11. It is a MEK kinase involved in pheromone response and in pseudohyphal/invasive growth pathways in yeast [65]. The Hsp90 co-chaperone Cdc37 is required for Ste11 maturation as it is the co-chaperone that generally interacts with Hsp90 protein kinase clients (Tables 5.1 and 5.2) [66]. A comprehensive study on the folding of Ste11 indicated that, in addition to Cdc37, the Hsp90 co-chaperones Sti1, Cpr7, and Cns1 are also involved in Ste11 maturation [67].

The protein kinase Gcn2 (Table 5.2) phosphorylates the α-subunit of translation initiation factor eIF2 (Sui2) in response to starvation. Phosphorylated eIF2α then becomes an inhibitor of eIF2β thus decreasing the rate of translational initiation by interfering with the eIF2 recycling pathway. Hsp90 binds and modulates the function of Gcn2, which requires co-chaperones Cdc37, Sti1, and Sba1 (Tables 5.1 and 5.2) [68].

Yeast gene *SWE1* (also known as *WEE1*) encodes a protein kinase involved in regulating cell cycle transition from G2 to M phase as mentioned above [69]. Phosphorylated Swe1 inhibits the kinase activity of the main cell cycle cyclin-dependent kinase Cdc28 by phosphorylating the conserved residue Tyr19 [70]. When tested in

the fission yeast strain *Schizosaccharomyces pombe*, overexpressed Swe1 triggered cell cycle arrest if Hsp90 was defective [71]. Swe1 was shown to co-immunoprecipitate with Hsp90 and the co-chaperones Sba1 and Cpr6/Cpr7 (Table 5.2).

The mitogen-activated protein kinase, MAP kinase, Slt2 (Table 5.2) was identified as a strong interactor of the yeast Hsp90 mutant, Hsp82 (E33A), in a two-hybrid screen [72]. Detailed analysis indicated that Hsp90 binds exclusively to the dually phosphorylated version Thr190/Tyr192 of the stress-activated form of Slt2. This was found when mutations in these two sites abolished interaction with Hsp90. Furthermore, in vivo analysis indicated that the Slt2 MAP kinase cascade requires the proper function of Hsp90.

Transcription factor Hap1 is another client of Hsp90 (Table 5.2). It modulates the expression of oxygen-dependent genes such as *CYC1* and *CYC7* in yeast [73]. The activity of Hap1 is regulated by heme binding. In the absence of heme, Hap1 forms a high-molecular-weight complex (HMC) that has low affinity for DNA. This HMC includes the molecular chaperones Hsp90, Hsp70, and Hsp40 [74]. Hsp70 plays a major role in Hap1 repression in the absence of heme while Hsp90 activates Hap1 when heme is present [75].

Mal63 is a transcription activator of genes involved in maltose metabolism including maltose permease and maltase. Yeast cells expressing a temperature-sensitive Hsp90 allele or lacking the co-chaperone Cpr7 were shown to down regulate Mal63 when grown in maltose limiting medium [76]. This suggests that Mal63 is a substrate of Hsp90 (Table 5.2).

6 Building the Global Hsp90 Interaction Network—Yeast

Hsp90 is involved in a very broad variety of processes with many of its functions still to be identified. However, it should be noted that despite the fact that Hsp90 is highly abundant under normal growth conditions and that its levels are elevated under environmental stress conditions, its functions are not related to promoting de novo protein folding or protein disaggregation but rather in the maturation or activation of proteins that are in a near-native state [77, 78]. In this sense, building a comprehensive Hsp90 chaperone network allows for a global view of this chaperone, its co-chaperones, and its clients.

In an application of an integrative proteomic approach, Zhao et al. [8] combined interaction data obtained from four different approaches. In the first approach, the authors carried out genome-wide systematic two-hybrid screens (2H screen) using ordered strain arrays to identify protein-protein interactions with full-length and different domains of Hsp90. In the second approach, they used the yeast library in which each open reading frame (ORF) is tagged at the C-terminus with a tandem affinity purification tag (TAP-tag) to carry out pulldowns of tagged ORFs and identify proteins interacting with Hsp90 using mass spectrometry (TAP screen). In the third approach, they screened for synthetic lethal interactions between a specific mutant allele of Hsp90 and members of a panel of about 4700 single yeast gene deletion

strains (synthetic genetic array, SGA, screen). In the fourth approach, they screened the deletion mutant strains for differential hypersensitivity to the Hsp90 inhibitor geldanamycin (GS screen). These efforts identified 627 putative Hsp90 interactors, representing about 10% of the yeast proteome (Fig. 5.3a).

From these studies, Zhao et al. [8] were able to elucidate new links between Hsp90 and transcriptional regulation, cell cycle, DNA processing, and cellular transport, among others. Furthermore, they were able to distinguish 84 Hsp90 clients identified in the two-hybrid and TAP screens that were also found to physically interact with 1 of 15 Hsp90 cofactors (Fig. 5.3b). Hence, this provided a first clue as to how client interaction with Hsp90 is stratified by the chaperone cofactors.

To highlight the strength of such a high-throughput analysis, in this study, the authors also identified new cofactors for Hsp90, namely Tah1 (Table 5.1) and its interactor Pih1. Tah1 and Pih1 were found to associate with the essential helicases Rvb1 and Rvb2 to form what was termed by the authors the R2TP complex. Hsp90-R2TP was subsequently shown to be involved in the assembly of box C/D small nucleolar ribonucleoprotein particles (snoRNPs) [79] required for pre-ribosomal ribonucleic acid (pre-rRNA) modification, phosphatidylinositol-3 kinase-related protein kinase (PIKK) signaling complexes [80], the telomerase reverse transcriptase (TERT) core complex [81], and RNA polymerase II [82–84]. Hence, the Zhao et al. study [8] allowed the characterization of new cellular pathways regulated by Hsp90 and its cofactors.

In another growth-based genetic interaction study, McClellan et al. [1] performed a genome-wide screen for negative genetic interactions between Hsp90 and target genes by examining the fitness of Hsp90-inhibited yeast cells, using the Hsp90 inhibitor macbecin II, versus Hsp90-non-inhibited yeast cells when these target genes were deleted. Furthermore, in order to gain insights into Hsp90 function in normal versus stress conditions, such experiments were conducted at 30 °C (normal yeast growth conditions) and 37 °C (heat stress conditions). The top 5% most growth-inhibited strains were selected as candidates for further analysis (Fig. 5.4).

By linking the identified genetic interaction pairs with both physical and genetic interaction pairs from the BioGRID database [85], the authors were able to construct the Hsp90 interaction network at the two temperatures. Under normal conditions in yeast, Hsp90 was found to be involved in protein secretion and trafficking. However, under heat shock conditions, this chaperone was found to be required for normal progression of the cell cycle, meiosis, and cytokinesis (Fig. 5.4). Further analysis using GO annotations [86] allowed the discovery of the modular nature by which the Hsp90 chaperone system operates. The two main groups that were found to form the Hsp90 network were (1) cellular trafficking and transport, and (2) regulation of the cell cycle. In a follow-up study, it was found that a total of 113 ORFs and 417 interactions are part of an Hsp90 functional network involved in the secretory pathway [1]. This network verified the notion that Hsp90 acts both in the exocytic and endocytic parts of the secretory pathway.

The Zhao et al. [8] and the McCellan et al. [1] analyses demonstrate the level of complexity of the Hsp90 network acting in many cellular pathways. The yeast Hsp90 network offers a significant level of completeness. This, therefore, becomes

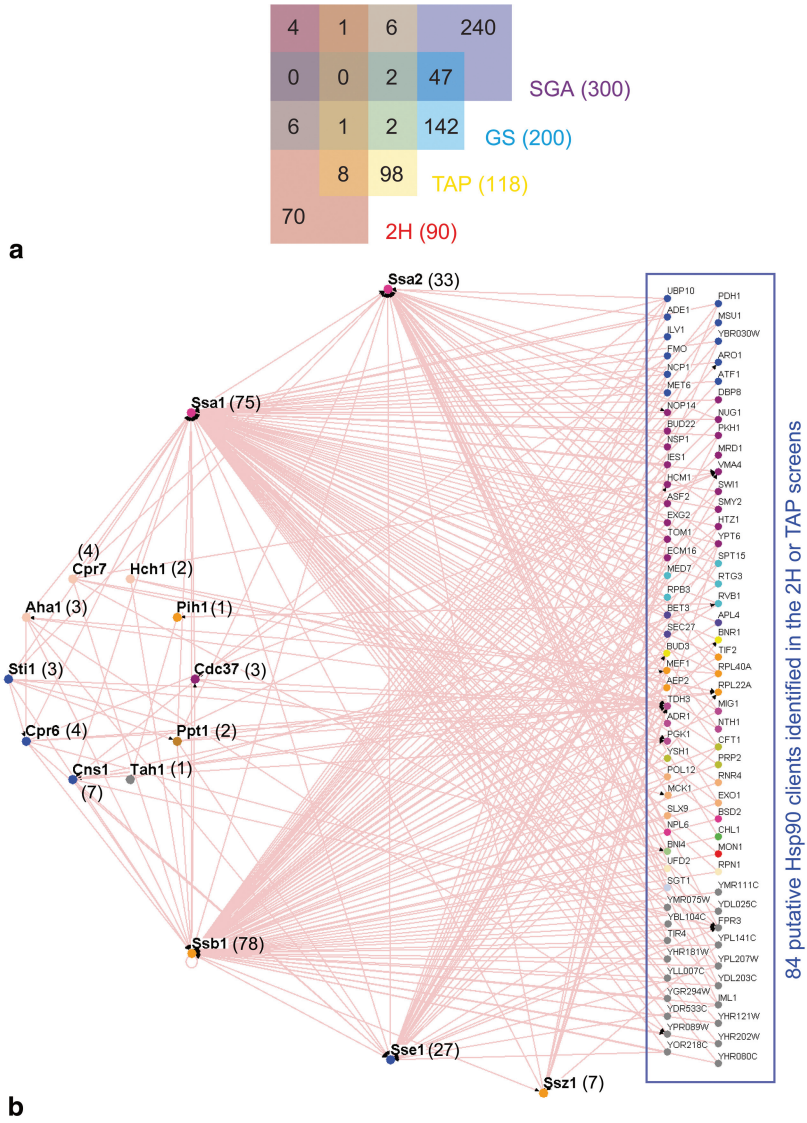

Fig. 5.3 Hsp90 interaction data from Zhao et al. [8]. **a** Venn diagram showing the overlap among proteins that were found to physically or genetically interact with Hsp90 using 2H, tandem affinity purification (TAP), synthetic genetic array (SGA), and geldanamycin screen (GS) screens. The total number of interactions for each method is indicated in brackets. **b** Interaction network of heat shock protein 90 (Hsp90) and its co-chaperones. Eighty-four putative clients identified in the 2H or TAP screens for Hsp90 were found to also interact with at least one of the 15 Hsp90 co-chaperones in TAP-tag pulldowns. The putative Hsp90 clients are grouped according to GO terms and are placed in the *blue rectangle*. *Lines* refer to TAP-based interactions. The numbers in brackets refer to the number of putative clients in the rectangle that interact with a given co-chaperone. (Modified from Zhao et al. [8]).

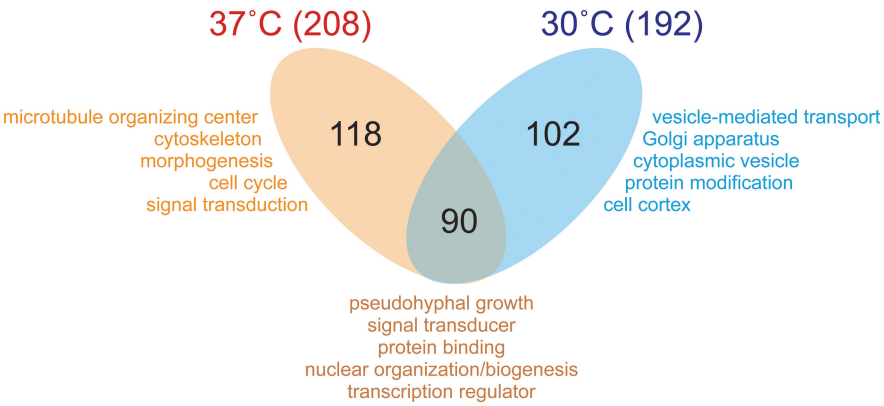

Fig. 5.4 Hsp90 interaction data from McClellan et al. [1]. Venn diagram showing the overlap among the Hsp90 chemical-genetic interactors identified at 30 and 37 °C. Total number of genes is indicated in brackets. The top five most significant GO slim compartment, function, and process categories are shown from data sets where genes were similarly enriched (*overlapping region*) and independently enriched for each condition (*non-overlapping regions*) (Data from McClellan et al. [1]).

an exceptional resource to tease apart the Hsp90 networks from many other organisms through comparative biology.

7 Building the Global Hsp90 Interaction Network—Mammalian Cells

In mammalian cells, most studies have typically mapped global cellular changes in protein levels upon treatment with an Hsp90 inhibitor. Although, such studies do not provide data for Hsp90-mediated protein-protein interactions, they do provide a global view of the cellular pathways that are directly or indirectly modulated by the chaperone.

By inhibiting Hsp90 ATPase activity using geldanamycin, Wu et al. [87] were able to identify protein level changes using different mass spectrometry-based methods including stable isotope labeling with amino acids in cell culture (SILAC) approach. They used four cancer cell lines K562 (blood), Colo205 (colon), Cal27 (head and neck), and MDAMB231 (breast) and identified about 1600 proteins that showed changes in levels upon drug treatment (Fig. 5.5a). They found that the rate of Hsp90 inhibition-induced protein downregulation correlates with protein half-life and that protein kinases have significantly shorter half-lives than other proteins.

In a similar study, Sharma et al. [88] used the Hsp90 inhibitor 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) on HeLa cells followed by SILAC analysis. They observed activation of the heat shock response and found that protein kinases and proteins involved in the DNA damage response were particularly affected by the inhibitor (Fig. 5.5b).

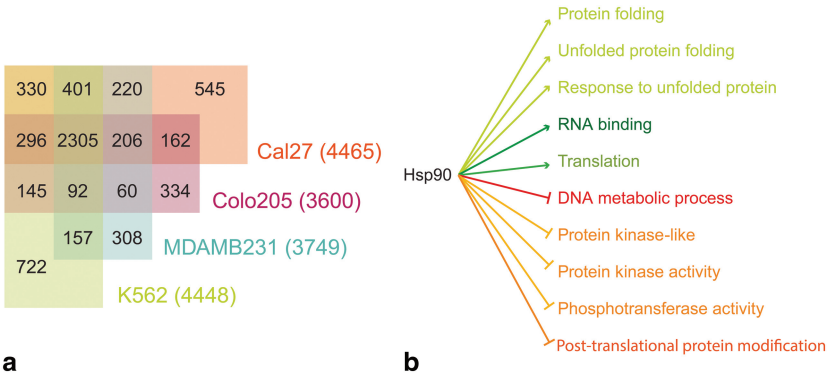

Fig. 5.5 Protein level changes upon heat shock protein 90 (Hsp90) inhibition in mammalian cells [87, 88]. **a** Summary of the number of proteins identified by Wu et al. [87] to significantly change levels upon Hsp90 inhibition in four human cancer cell lines: Cal27 (*head and neck*), Colo205 (colon), MDAMB231 (breast), and K562 (blood). Total numbers of proteins are indicated in brackets. **b** Statistically significant annotated terms (KEGG, GO, PFAM, and SCOP) found for proteins whose levels significantly changed upon treatment of HeLa cells with the Hsp90 inhibitor 17-DMAG ((dimethylaminoethylamino)-17-demethoxygeldanamycin) as determined by Sharma et al. [88]. Upregulated groups are shown in *green*, downregulated groups are in *red*. Groups that correspond to the same pathway are color-coded in different shades of *green* or *red*.

By performing a quantitative high-throughput analysis, Taipale et al. [89] were able to measure the interaction of Hsp90 and the co-chaperone Cdc37 with human protein kinases, transcription factors and ubiquitin ligases *in vivo*. They found interactions between Hsp90 and almost 400 client proteins with kinases making up the largest proportion of interactors. Cdc37 was found to serve as an adaptor to provide specificity for Hsp90 interaction with kinases. They were able to show that Hsp90 clients within the kinase family can be differentiated from non-clients on the basis of their intrinsic stability. They propose that Hsp90 kinase clients are generally unstable in their fully folded state and this makes them more prone to recognition by Hsp90 and Cdc37. Transcription factors were found to be chaperoned by Hsp90 in a similar way as kinases but represent only a small fraction of Hsp90 clients.

All studies clearly suggest that kinases are the major group of Hsp90 clients in mammalian cells and that they are destabilized upon Hsp90 inhibition.

8 Building the Co-chaperone Interaction Network—The Case of Sba1

Building the interaction network for Hsp90 co-chaperones has so far only been done for yeast Sba1 (yeast ortholog of mammalian p23, Table 5.1). Echtenkamp et al. [42] carried out an SGA analysis of *SBA1* yeast deletion strain against a deletion library to identify parallel or compensatory pathways affected by Sba1. They found that Sba1 genetic interactors were enriched in signal transduction, protein

catabolism, cell budding, DNA metabolism, cellular respiration, and vesicle-mediated transport. Sba1 was also found to serve as a negative regulator of vesicle transport.

Interestingly, the data suggest that Sba1's main function in the cell might be independent of Hsp90 since only ~25% of the 348 total Sba1 interactors overlap with known Hsp90 interactors. Nevertheless, Sba1 and Hsp90 appear to function in the same biological pathways but interacting with clients at different points along those pathways. For example, Sba1 seems to modulate protein transport regulating protein mannosylation in the Golgi, whereas Hsp82 affects that pathway by interacting with vesicle-tethering complexes [1, 42]. In a similar scenario, the authors show that both Hsp82 and Sba1 act on cell mobility by interacting with different clients. By intersecting at individual proteins, protein complexes, and protein pathways, Sba1 likely established a relationship with Hsp90 and cellular processes that systematically expand the reach of the Hsp90 system.

9 Hsp90 Interaction Networks in Pathogens

Hsp90 has gained much popularity as a target for treating various infectious diseases in addition to cancer. It is involved in many important cellular processes of intracellular protozoans and other important human pathogens [90, 91]. Building a chaperone network to understand the biology of these pathogens is a powerful tool for drug discovery efforts [10].

In *Candida albicans* (the most prevalent human fungal pathogen), Hsp90 regulates not only drug resistance but also morphogenesis and virulence, making it an ideal organism to study Hsp90 functions in pathogenesis [91]. Diezmann et al. [91] performed a chemical-genetic screen using geldanamycin as an Hsp90 inhibitor on mutant strains from a homozygous transposon insertion library. This was done under a variety of stress conditions, such as: NaCl (osmotic stress), tunicamycin (causes the unfolded protein response in the endoplasmic reticulum), azole fluconazole (targets the cell membrane), chinocandin caspofungin (targets the cell wall), and growth at 37 or 41 °C (heat stress). A network was built with Hsp90 targets as nodes linked to the associated genetic interaction condition. In addition to genetic interactivity, the dependence of certain target genes on Hsp90 was determined by measuring protein or transcript levels upon Hsp90 depletion. The authors found that the networks obtained under different conditions are quite different with little overlap.

A comparison of the genetic interactions between *C. albicans* and *S. cerevisiae* revealed that certain interactions are conserved between the two fungi and that the conservation was dependent on the experimental conditions employed. The authors found that, for interactors in *C. albicans* that have homologs in *S. cerevisiae*, only 17% of *C. albicans* Hsp90 interactors were conserved in *S. cerevisiae*. This indicates that there has been considerable rewiring of the Hsp90 network over evolution. However, although the inferred interactions were different between the two organisms, they both retained similar proportions of their genome associated with Hsp90.

In the malarial parasite *Plasmodium falciparum*, a chaperone network was inferred using computational approaches [92]. This parasite undergoes frequent episodes of heat shock during febrile periods, which is a clinical hallmark of malaria. It is therefore reasonable to assume that chaperones play an important role in the parasite's adaptation. Hsp90 has been shown to be essential for parasite viability. The analysis was performed by interolog mapping of a human network derived from both the human protein reference database (HPRD) and from predicted malaria-human orthologs using BLASTP. Interologs are protein-protein interactions that are conserved across species. This network was then combined with protein-protein interactions from a yeast two-hybrid screen for malarial proteins to construct the chaperone network for this parasite. The network allowed the authors to perform various putative predictions on chaperone function during parasite development. For example, they were able to find an Hsp40 molecule that acts in concert with Hsp90 in membrane protein trafficking.

The networks described for *C. albicans* and *P. falciparum* highlight the importance of Hsp90 in pathogen drug resistance, morphogenesis, stage development, and virulence. More importantly, they provide clues towards selecting Hsp90 interactors that may serve as putative drug targets. These analyses reveal again the extraordinary versatility of Hsp90 activities and its involvement in multiple cellular pathways. They also demonstrate that there is still much to be discovered about this essential chaperone system.

10 Concluding Remarks

Hsp90 is a central molecular chaperone for maintaining protein homeostasis. Its multiple co-chaperones and its functional interaction with several other chaperones makes mapping the interaction network of Hsp90 a requirement for understanding its cellular activities. Currently, the Hsp90 interaction network has been extensively studied only in yeast and future studies have to concentrate on mapping the network in different mammalian cell lines under normal and disease states. This is especially important due to the fact that Hsp90 is an anticancer drug target. Furthermore, mapping the Hsp90 network in different pathogens will help in drug discovery efforts targeting these pathogens by targeting Hsp90. Multiple proteomic approaches have to be employed to elucidate such networks. The techniques currently being employed are still under development and will require further improvement to be able to be implemented by the many groups working on Hsp90. Gathering many types of high-throughput data simultaneously (genetic and physical interactions; localization of small molecules, RNA, lipids, proteins) could be a challenging task but is absolutely required to allow for the generation of an integrated network that more clearly reflects the plethora of functions of this chaperone.

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