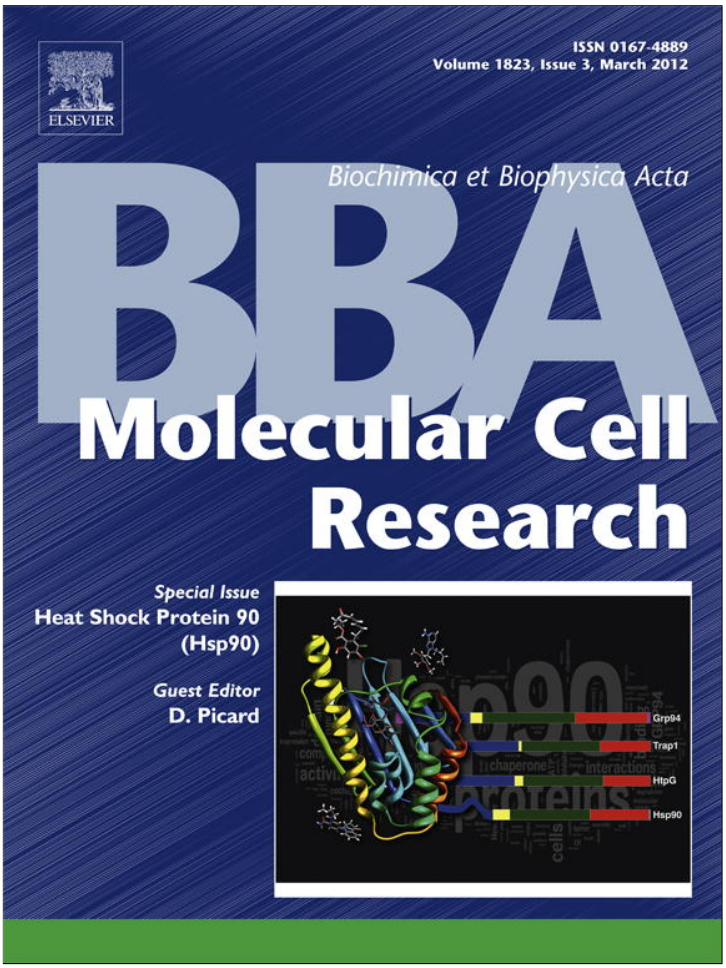

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Review

The role of Hsp90 in protein complex assembly☆

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ABSTRACT

Hsp90 is a ubiquitous and essential molecular chaperone that plays central roles in many signaling and other cellular pathways. The in vivo and in vitro activity of Hsp90 depends on its association with a wide variety of cochaperones and cofactors, which form large multi-protein complexes involved in folding client proteins. Based on our proteomic work mapping the molecular chaperone interaction networks in yeast, especially that of Hsp90, as well as, on experiments and results presented in the published literature, one major role of Hsp90 appears to be the promotion and maintenance of proper assembly of protein complexes. To highlight this role of Hsp90, the effect of the chaperone on the assembly of the following seven complexes is discussed in this review: snoRNP, RNA polymerase II, phosphatidylinositol-3 kinase-related protein kinase (PIKK), telomere complex, kinetochore, RNA induced silencing complexes (RISC), and 26S proteasome. For some complexes, it is observed that Hsp90 mediates complex assembly by stabilizing an unstable protein subunit and facilitating its incorporation into the complex; for other complexes, Hsp90 promotes change in the composition of that complex. In all cases, Hsp90 does not appear to be part of the final assembled complex. This article is part of a Special Issue entitled:Heat Shock Protein 90 (HSP90).

1. Overview of Hsp90 structure and activity

In cell biology, the whole is always more complex than the simple sum of its parts. Based on this principle, spatial and temporal assembly and disassembly of multiprotein complexes is crucial for the viability of cellular organisms. One of the important questions in cellular biology is how numerous protein–protein interactions are regulated in the crowded cellular environment. Recent findings suggest that molecular chaperones occupy a central place in the assembly of multimeric complexes since they are ideally suited for helping interacting proteins acquire proper folding and at the same time chaperones mitigate the risk of unspecific interactions that eventually lead to aggregation [1–6]. Heat shock protein 90 (Hsp90) is a highly conserved molecular chaperone present from bacteria to mammals. In eukaryotes, Hsp90 is essential for cell viability under all growth conditions tested [7,8]. Hsp90, together with Hsp70 and cochaperones, make up the Hsp90 chaperone machineries which stabilize and activate more than 200 proteins in mammalian cells. These proteins are referred to as Hsp90 clients and typically play essential roles in constitutive cell signaling and adaptive responses to stress [9]. Furthermore, recent systematic studies on Hsp90 [10–12] have suggested that the number of client proteins might be even larger. However, few in vivo roles of Hsp90 have been clearly demonstrated.

Hsp90 is a conformationally dynamic dimeric protein. Each monomer can be divided into a highly conserved N-terminal domain that contains a unique ATP-binding pocket and cochaperone interacting motifs, a Middle domain that contains binding sites for client proteins and cochaperones, and a C-terminal domain that harbors the dimerization motif. The N-terminal domain is connected to the Middle domain by a charged linker; phosphorylation of this linker region modulates Hsp90 activity [13–15]. ATP binding to the N-terminal domain and its subsequent hydrolysis by Hsp90 drives a conformational cycle that is essential for chaperone activity [16]. Two extreme conformational states for the Hsp90 dimer have been observed [17,18]. 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 structure, and a 'relaxed' state in which the N-terminal domains are dissociated and the dimer has a V-shaped structure. Several other conformational states for Hsp90 have also been observed highlighting the complexity of the chaperone functional cycle; nevertheless, Hsp90 exhibits a generally conserved three-state conformational cycle: apo, ATP, and ADP [19].

In mammalian cells, Hsp90 is present in the cytoplasm (Hsp90α and Hsp90β), as well as, in the mitochondria (TRAP1) and endoplasmic reticulum (Grp94) [20,21]. Hsp90 has also been found on the surface of different cancer cells, as well as, secreted into the extracellular matrix [22,23]. This suggests many different roles for Hsp90 in

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the different compartments and, hence, in principle, Hsp90 can assist in protein folding and complex formation in every cellular compartment.

Hsp90 cellular function is regulated by more than 20 cochaperones that have diverse effects on its biochemical activities. For example, cochaperones regulate Hsp90 by modulating its rate of ATP hydrolysis (Aha1, Cdc37, p23), conformational flexibility (p23, Sgt1), and the binding of specific substrates or higher-order protein complex assembly (HOP, Cdc37, Sgt1) [24–29]. In addition, cochaperones can modulate Hsp90 function by catalyzing ubiquitination or dephosphorylation of client proteins [30,31]. Hsp90 also cooperates with other chaperones for protein folding, mainly Hsp70 and Hsp40 chaperones. The assembly of Hsp90/Hsp70 machinery requires binding of both chaperones to Hop/p60 (Sti1 in yeast) — a cochaperone with three tetratricopeptide repeat (TPR) domains [32,33]. It is proposed that the N-terminal domain of Hop binds to the C-terminal EEVD motif of Hsp70, and the two C-terminal TPR domains bind to the C-terminal EEVD motif of Hsp90, hence, forming a platform for Hsp70–Hsp90 interaction [34,35]. The Hsp90–Hsp70–Hop complex is a central intermediate in the Hsp90 functional cycle.

Based on our recent comprehensive analysis of the physical interaction network of all the yeast chaperones [36], as well as, on our prior multi-method high-throughput mapping of the interaction network of yeast Hsp90 [11], we have suggested that one major role of molecular chaperones is to promote and maintain the proper assembly of protein complexes. By virtue of its activity, Hsp90 is well-suited to coordinate the assembly of many protein complexes because it functions at late stages of protein folding with clients in near-native states, whereas other chaperones generally function at earlier stages and facilitate the folding and maturation of nascent proteins.

This review highlights several examples in which Hsp90 has been found to play a key role in protein complex formation. We will discuss the role of Hsp90 in the assembly of the following seven complexes: snoRNP, RNA polymerase II, phosphatidylinositol-3 kinase-related protein kinase (PIKK), telomere complex, kinetochore, RNA induced silencing complexes (RISC), and 26S proteasome.

2. Role of Hsp90 in box C/D snoRNP complex assembly and pre-rRNA processing

Eukaryotic ribosomes contain four ribosomal RNAs (5S, 5.8S, 18S, and 25/28S) and about 75 different ribosomal proteins [37]. Ribosome synthesis takes place mainly in the nucleolus. In *Saccharomyces cerevisiae*, for example, there are about 150 rDNA repeats of ~9.1 kbp, each of which is tandemly arranged on chromosome XII. A single unit of rDNA contains two genes: the 5S and 35S pre-rRNA genes. The 5S pre-rRNA is transcribed by RNA polymerase III, while the 35S pre-rRNA is transcribed by RNA polymerase I. The 35S pre-rRNA is then extensively modified and processed to generate 5.8S, 18S, and 25S rRNAs (Fig. 1). Over 100 trans acting factors are involved in these activities including: small nucleolar ribonucleoprotein particles (snoRNPs) containing small nucleolar RNAs (snoRNAs) and conserved protein components, RNA helicases,

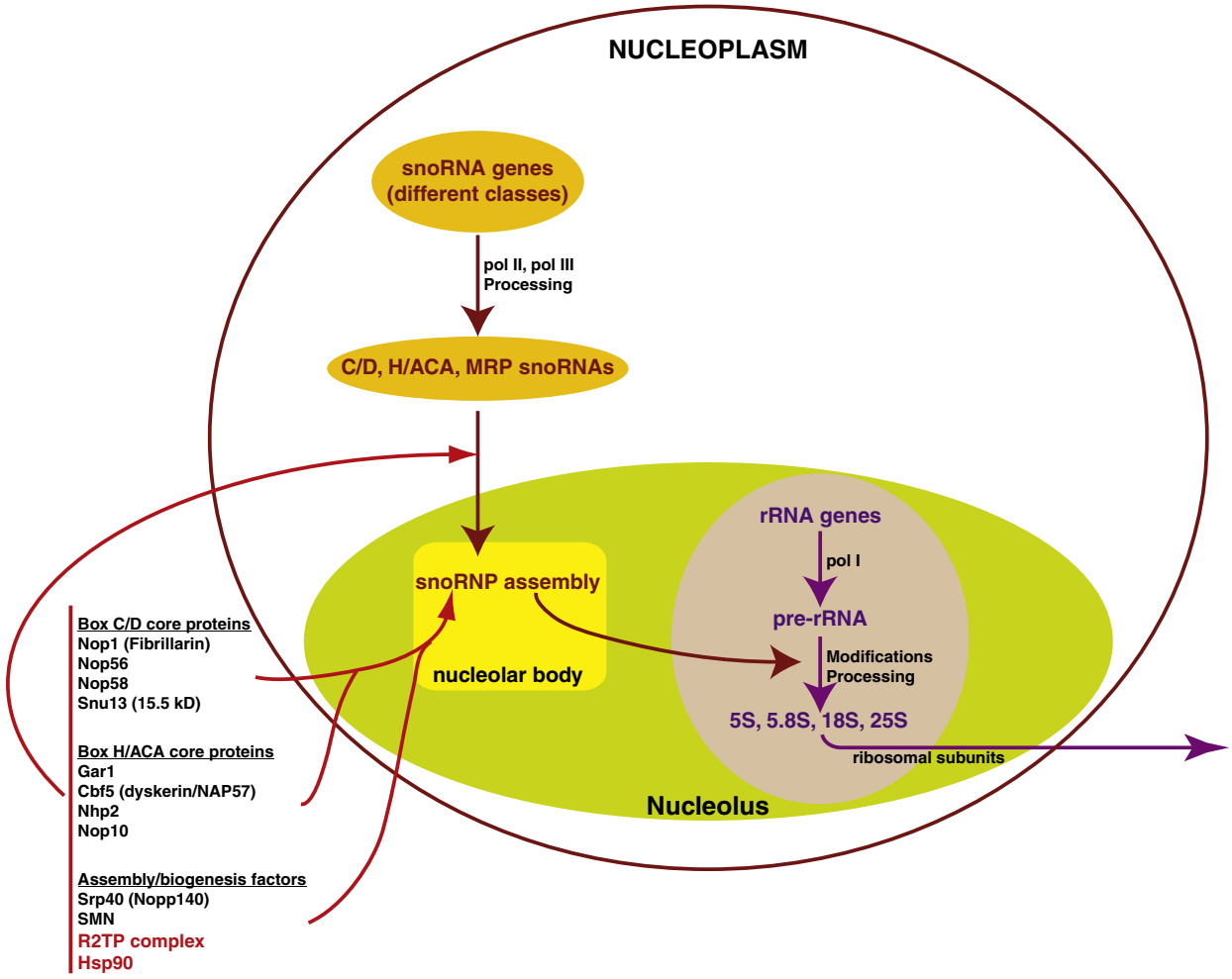

Fig. 1. Overview of snoRNA biogenesis. The schematic refers to yeast cells. Nucleolar bodies in yeast are thought to correspond to mammalian Cajal bodies. However, Cajal bodies are localized in the nucleoplasm outside the nucleolus. Refer to the text for details.

endonucleases, exonucleases, exosome components, nucleolar proteins, assembly factors, nucleocytoplasmic transport factors, and others. Hence, vast resources are dedicated for the fundamental process of ribosome biogenesis.

snoRNAs are grouped into three classes based on conserved structural elements and sequence motifs: MRP snoRNA (one member in yeast), box C/D snoRNAs (46 known members in yeast), and box H/ACA snoRNAs (29 known members in yeast). snoRNPs from the different classes have specific core proteins. The core protein components for the box C/D snoRNPs are Nop1 (fibrillarin), Nop56, Nop58, and Snu13 (15.5 K); while the core protein components for the box H/ACA snoRNPs are Gar1, Cbf5 (dyskerin/Nap57), Nhp2, and Nop10. The general principle of function of the snoRNPs relies on base pairing of the snoRNA with a specific site on the pre-rRNA and subsequent modification of the pre-rRNA by the snoRNP protein components. Box C/D snoRNPs are mainly involved in 2′-O-ribose methylation catalyzed by Nop1, which is structurally similar to methyltransferases. Box H/ACA snoRNPs are primarily involved in pseudouridylation of the pre-rRNA catalyzed by Cbf5, which is structurally similar to pseudouridine synthases.

The biogenesis of snoRNPs is a highly complex and dynamic process that is still poorly understood [38]. Most yeast snoRNAs are transcribed from independent genes, mostly by RNA polymerase II. However, mature snoRNAs are typically generated by post-transcriptional processing from larger transcripts (pre-snoRNAs) [39]. Some snoRNAs are processed from polycistronic transcripts containing as many as nine different snoRNAs [40]. There are 17 polycistronic snoRNAs in yeast, all of which are of the box C/D type. However, many vertebrate snoRNAs and eight yeast snoRNAs (six box C/D: U18, U24, snR38, snR39, snR54, snR59, and two box H/ACA: snR44, snR191) are excised from introns of mRNAs.

Pre-snoRNAs are synthesized in the nucleoplasm (Fig. 1). Early processing steps and the interaction of snoRNAs with core proteins are also thought to occur in the nucleoplasm. In vertebrates, some processing and assembly also occurs in Cajal bodies [38]. Cajal bodies are nuclear domains that are biochemically and structurally linked to the nucleolus and contain many components of different transcription and RNA-processing machinery [41]. The nucleolar body in yeast shares similarities with the vertebrate Cajal body and may be involved in the assembly and trafficking of box C/D snoRNAs [42,43]. The yeast nucleolar body is localized inside the nucleolus [42] (Fig. 1). It has been proposed that the core snoRNP proteins are recruited to their respective snoRNAs co-transcriptionally [44,45], although this remains controversial.

For box C/D snoRNAs, Snu13 is proposed to bind first to the snoRNA followed by Nop1, Nop56, and Nop58 [46]. Other proteins associate transiently with the snoRNPs to promote assembly and trafficking. These include the phosphoprotein Srp40 (Nopp140), which might facilitate transport and retention of snoRNPs [42,47], and the survival of motor neurons protein (SMN), which directly interacts with the box C/D core protein, fibrillarin, and box H/ACA core protein, Gar1 [48]. Our work as well as that of others has suggested that Hsp90 is also involved in snoRNP assembly.

To investigate the global role of Hsp90 inside the cell, we carried out comprehensive screens using the latest proteomic methods to identify novel interacting partners of yeast Hsp90 [11]. These efforts were based on complementary physical and genetic experimental strategies. The data indicated that Hsp90 physically and/or genetically interacts with about 10% of the yeast proteome, clearly establishing Hsp90 as a central hub in many cellular pathways. Several uncharacterized candidates from the proteomic study were further investigated. Two of these proteins, which we termed Tah1 (YCR060w — contains two TPR motifs) and Pih1 (YHR034c, also now known as Nop17 — has no known motifs), showed very interesting biochemical characteristics that suggested a novel mechanism by which Hsp90 might exert its influence on pre-rRNA processing in the cell. It was shown that Pih1 interacts with the C-terminus of Tah1 and of Hsp90, and that full length Tah1 is required to bind to the C-terminus of Hsp90 (Fig. 2). Furthermore, Tah1 and Pih1 were found to form a complex with the essential and highly conserved helicases Rvb1 and Rvb2; we termed the Rvb1–Rvb2–Tah1–Pih1 complex R2TP. Rvb1/2 were found to form a heterohexameric complex, with Pih1 directly binding to that complex, while Tah1 is recruited to the complex through its interaction with Pih1 (Fig. 2). The R2TP complex is highly conserved and was shown to be present in human cells [1,49,50].

Subsequently, we demonstrated that Hsp90 and Tah1 actually work to stabilize Pih1. In vitro, Pih1 tends to aggregate; Hsp90 was able to disaggregate Pih1 in a nucleotide-dependent manner (Fig. 2). This novel disaggregation activity of Hsp90 was not reported before, and the activity seems to be enhanced by Tah1. Furthermore, depletion of Tah1 in the cell resulted in the degradation of Pih1. In addition, in cells that had diminished levels of Hsp90, the box C/D core protein complex Snu13–Nop1–Nop56–Nop58 was not properly formed due to reduced Snu13 levels in the complex, indicating a possible role of Hsp90 in box C/D core protein complex formation/maintenance. Based on data obtained from synthetic lethal screens and microarray analyses, in addition to Northern analyses on cells deleted or depleted of R2TP proteins [51], a role for the R2TP complex in box C/D snoRNA accumulation was demonstrated. In addition, involvement of R2TP complex in snoRNP assembly in yeast was further supported by the finding that Pih1 interacts with box C/D core protein, Nop58 and that *PIH1* deletion affects pre-rRNA processing [52,53]. Since it was found that Hsp90 works to stabilize Pih1 and box C/D core protein complex, Hsp90 was expected to also have an effect on box C/D snoRNA accumulation and eventually on pre-rRNA processing. Microarray studies and Northern analyses on cells depleted of Hsp90 showed the accumulation of 35S pre-rRNA, which supported such expectations.

Based on the above results, a model is proposed based on the stabilization of protein complexes mediated by Hsp90 (Fig. 2). Hsp90 and Tah1 bind and stabilize Pih1, probably resulting in a transient formation of an Hsp90–Tah1–Pih1 ternary complex (Fig. 2). Tah1 and Pih1 are then transferred to the Rvb1/2 complex resulting in the formation of the R2TP complex. Hsp90 and R2TP are then involved in biogenesis/assembly of box C/D snoRNPs (Fig. 2). Neither Hsp90 nor R2TP becomes part of the mature snoRNP complex.

The R2TP complex was also found in human cells where it usually associates with a Prefoldin-like module to form a larger 11 subunit R2TP/Prefoldin-like complex [1,3,4,49,54]. The mammalian proteins equivalent to yeast Rvb1, Rvb2, Tah1 and Pih1 are termed RuvBL1 (also known as pontin and many other names), RuvBL2 (also known as reptin and many other names), RPAP3 (also known as hSpagh), and PIH1D1, respectively. The model proposed in Fig. 2 also seems to hold for mammalian cells [1].

3. Role of Hsp90 in RNA polymerase II assembly

RNA polymerases are key multisubunit cellular enzymes involved in transcription. The functional RNA polymerase II complex consists of 12 subunits, termed Rpb1 to 12 [55], and is involved in the synthesis of mRNAs and noncoding RNAs (e.g. the snoRNAs discussed above). Hsp90 together with the R2TP/Prefoldin-like complex were found to be required for the assembly of this polymerase (Fig. 3A) without being part of the mature complex.

Rpb1 and Rpb3 were found to accumulate in the cytoplasm when assembly of the polymerase was prevented by the compound α-amanitin [3]. The compound binds to the Rpb1 subunit with high affinity and triggers its degradation [3,56] resulting in the disassembly of the polymerase. Consistently, inhibition of Crm1-dependent nuclear export of Rpb3 by leptomycin B or depletion of any subunit of the RNA polymerase II results in Rpb1 accumulation in the cytoplasm. Based on these results, the authors suggested that Rpb1 must be incorporated into the fully assembled RNA polymerase II enzymes in the cytoplasm before its nuclear import [3]. Further experiments aimed at the characterization of the assembly intermediates of the

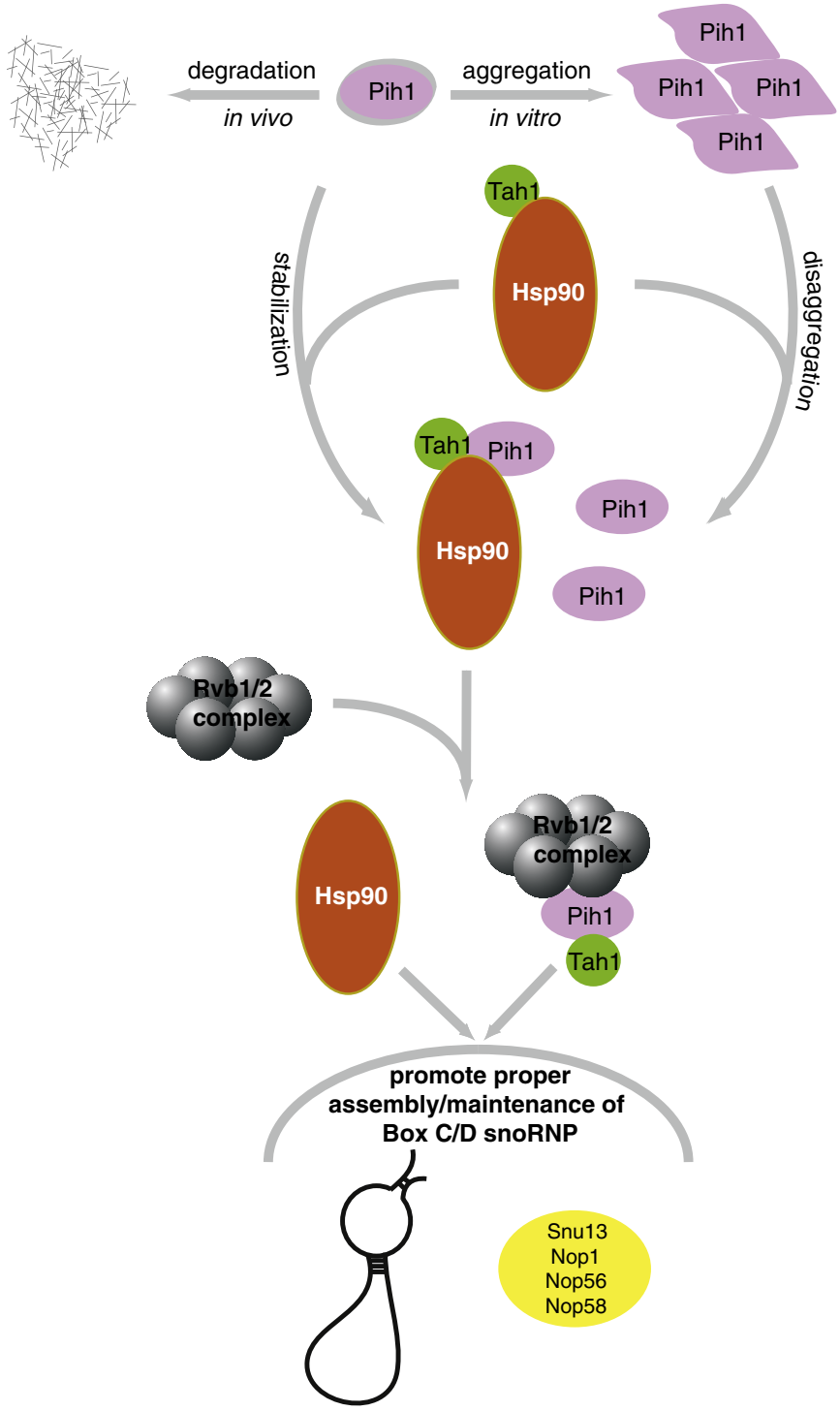

Fig. 2. The role of Hsp90 in mediating the assembly of the R2TP and snoRNP complexes. A cartoon representation of the current working model for the function of Hsp90/R2TP. It is proposed that Hsp90 and Tah1 bind and stabilize Pih1 against degradation or aggregation, resulting in a transient formation of an Hsp90–Tah1–Pih1 ternary complex. Tah1 and Pih1 are then transferred to the Rvb1/2 complex resulting in the formation of the R2TP complex. Hsp90 and R2TP are then involved in the proper assembly of box C/D snoRNPs. The figure is modified from reference [51].

polymerase by MS-based quantitative proteomics indicated the presence of a cytoplasmic complex containing subunits Rpb1 and Rpb8 associated with the R2TP/Prefoldin-like complex (Fig. 2). These results are consistent with another work which mapped the RNA polymerase II-associated complexes [54]. It was also shown that Hsp90 stabilizes free Rpb1 [3].

A model is proposed [3] whereby RPAP3 binds to unassembled Rpb1 and then delivers Rpb1 to Hsp90, hence, allowing Hsp90-RPAP3 to coordinate the assembly of RNA polymerase II in the cytoplasm (Fig. 3A). However, the exact mode of action is still unknown. The authors suggested that a possible role for Hsp90 could be to maintain Rpb1 in a conformation compatible with assembly and to mitigate the association with incorrect partners.

4. Role of Hsp90 in the assembly of PIKK complexes

Tel2 is an essential protein that has been implicated in many distinct cellular processes including DNA repair, DNA damage checkpoint,

telomere maintenance, the regulation of biological clock, and signal transduction [57–61]. The discovery that Tel2 binds to and is required for stable expression of all six members of the phosphatidylinositol-3 kinase-related protein kinase family proteins in human cells (ATM, ATR, DNA-PKcs, mTOR, SMG1, and TRRAP) raised the possibility that Tel2 is a universal regulator of PIKKs [62]. PIKKs are Ser/Thr-protein kinases that respond to various stresses [63] and are similar in sequence to phosphatidylinositol-3 kinases (PI3Ks), hence their name. ATM, ATR, and DNA-PKcs are involved in DNA damage response [64], mTOR is a nutrient-regulated kinase that controls a wide variety of pathways involved in cell growth and metabolism [65], SMG1 regulates nonsense-mediated mRNA decay as part of the mRNA surveillance complex [66], and TRRAP is part of a multiprotein co-activator complex possessing histone acetyltranferase activity important for the transcriptional activity of c-Myc and other transcription factors [67].

PIKKs have been found in complex with RuvBL1and RuvBL2 [68]. Furthermore, experiments showed that treatment of cells with Hsp90 inhibitor 17-AAG results in loss of ATR, ATM, and DNA-PKcs at the protein level [4,69] suggesting that Hsp90 is required for the maturation and stability of these kinases. It was suggested that Tel2 functions to link the activity of Hsp90 and the R2TP/Prefoldin-like complex with the assembly and/or stabilization of the PIKKs and that PIH1D1 is required for the recognition and binding of constitutively phosphorylated Tel2 [4] (Fig. 3B). It was further demonstrated that the formation of three PIKK complexes, namely TORC1, TORC2 and ATR, is impaired when either Tel2 was deleted or when Hsp90 was inhibited [69]. Specifically, deletion of Tel2 or inhibition of Hsp90 impaired the association of newly synthesized mTOR with its binding partners in TORC1 and TORC2 complexes. Under the same conditions, ATR binding to ATRIP which is required for ATR signaling was also inhibited [70] (Fig. 3B).

However, the precise mode of action of Hsp90 and Tel2 in PIKK complex assembly will require detailed biochemical and structural data. Possible modes could be that Tel2 helps recruit PIKKs to the Hsp90-R2TP/Prefoldin-like complexes or that Tel2 tunes the rate of Hsp90 ATP hydrolysis in a manner that is optimal for this specific class of substrates, similar to the action of Hsp90 cofactor Cdc37 in the activation of Ser/Thr kinases [71]. On the other hand, Hsp90-R2TP/Prefoldin-Tel2 chaperone system may prevent the aggregation of newly synthesized PIKKs until they are incorporated into functional complexes.

5. Role of Hsp90 in telomere complex assembly

Telomeres are heterogeneous nucleoprotein assemblies at eukaryotic chromosome termini that serve both to protect the chromosome ends from damage and to extend DNA length following replication. To accomplish these activities, various proteins are nucleated at telomeric DNA to form distinct structures including capping and extending complexes [11,72]. Genetic analysis in budding yeast revealed a number of genes which encode critical components of the telomere, including *EST2*, *STN1*, *TEN1*, and *CDC13*. Est2 is a reverse transcriptase subunit that is responsible for extending telomeric DNA, while Stn1 and Ten1 function to cap the chromosome ends. Cdc13 promotes both DNA extension and protection in in vivo studies supporting the model in which Cdc13 recruits and stabilizes both Est2-extending and Stn1/Ten1-capping structures [73–75].

Various studies implicated Hsp90 in the regulation of telomerase activity, stability, and localization [76–79]. For example, in budding yeast, the $HSC82^{S481Y}$ mutant strain had shortened telomeric DNA but normal telomerase activity in vitro [80]. Notably, the Hsp82 chaperone was found to be a high-copy suppressor of the two mutants with telomere defects, *cdc13-1* and *stn1-157* [81] highlighting the link between Hsp90 and telomere capping and extending complexes. The molecular mechanism explaining the role of Hsp90 in telomere organization in budding yeast was elucidated by DeZwaan and colleagues [2]. Specifically, it was demonstrated that, in S-phase cells, Hsp90 orchestrates the alternation between telomere capping complex Cdc13–Stn1–Ten1–DNA and telomere extension complex Est1–Est2–Est3–Cdc13–DNA. Hsp90 binding triggers the dissociation of the capping complex from the telomeric DNA by inhibiting the binding of Cdc13 to DNA. At the same time, Cdc13 ability to associate with the DNA-bound telomerase is not affected by Hsp90 and this promotes the assembly of the telomere extension complex (Fig. 3C). The ability of Hsp90 to induce DNA dissociation of capping (Cdc13) complexes and to promote the association of extension (telomerase) complexes demonstrates how Hsp90 facilitates telomere maintenance by promoting transitions between different functional complexes at telomeric DNA.

The regulation of telomerase function by Hsp90 is also conserved in humans. The catalytic subunit of human telomerase, hTERT, was shown to associate with the Hsp90–p23 chaperone complex [82,83]; furthermore, the telomerase activity in mammalian cells can be inhibited by geldanamycin [84]. Interestingly, the integrity of hTERT–Hsp90–p23 complex is required for nuclear localization of hTERT since the dissociation of the p23 cofactor from Hsp90-hTERT results in the cytoplasmic retention of hTERT [85].

6. Role of Hsp90 in kinetochore assembly

The kinetochore is a large and dynamic protein complex that attaches the chromosomes to spindle microtubules during cell division, thus, allowing the spindle to pull the chromosomes apart. The kinetochore complex assembles on the centromere and, in budding yeast, this assembly occurs in a hierarchical manner whereby the centromere binding complex 3 (CBF3) is first assembled before it can bind to sequence-specific elements on centromeric DNA (CEN DNA) and recruit additional complexes that form a single microtubule binding site [86,87].

CBF3 is a large multisubunit protein complex that makes both sequence-specific and nonspecific contacts with the CDE III element of centromeric DNA; CBF3 consists of four protein components: Cep3, Ctf13, Skp1, and Ndc10 [86,88,89]. The activity of Ctf13 depends on Skp1 and the Hsp90 cochaperone Sgt1. Sgt1 consists of an N-terminal TPR domain; a middle 'CHORD and Sgt1' (CS) domain related to the β-sandwich domain of small heat-shock proteins such as α-crystallin and the Hsp90 cochaperone p23/Sba1; and a C-terminal Sgt-specific (SGS) domain [29]. It has been proposed that Hsp90 activity is required for the assembly of the CBF3 complexes [90,91].

It is proposed that Hsp90 mediates the formation of a pre-activation complex consisting of Ctf13 bound to Cep3, Skp1, and Sgt1 (Fig. 3D). ATP-bound Hsp90 is found to interact with Sgt1 and to stabilize it in a closed inhibited conformation in which the Sgt1 N-terminal TPR domain interacts with the C-terminal SGS domain (Fig. 3D). Upon ATP hydrolysis, ADP-bound Hsp90 promotes the formation of the open Sgt1 conformation allowing Skp1p and Ctf13p to interact with Sgt1. Upon nucleotide exchange, ATP-Hsp90 is formed and Sgt1 returns to its closed state resulting in the release of Sgt1 and Hsp90 and the binding of Ndc10 to Ctf13, hence, completing the assembly of the CBF3 complex which then forms high affinity interactions with CEN DNA (Fig. 3D).

Based on the model, Hsp90 is required to maintain the transient interaction between Sgt1 and Skp1/Ctf13. Consistently, mutations that, for example, stabilize the interaction between Sgt1 and Skp1 compromise CBF3 assembly and interfere with normal cell growth. Notably, Skp1 is also a component of the SCF (Skp1–Cullin–F–box protein complex) E3 ubiquitin ligase complex that regulates the turnover of CBF3 complex. Interestingly, coupling of the Hsp90 and Sgt1 is also required for the assembly of the SCF ubiquitin ligase complexes. Hence, Hsp90–Sgt1 balances CBF3 complex assembly with its turnover [29].

The role of Hsp90–Sgt1 complex in the formation of functional kinetochores is conserved in mammalian cells. In HeLa cells, Hsp90–Sgt1 interacts with and stabilizes the Mis12 complex, required for the assembly of a large fraction of the kinetochore [92]. Hsp90–Sgt1 mediated assembly of Mis12 complexes is necessary for the timely formation of high-affinity MT-binding sites, whereas the turnover of Mis12 complexes is required to ensure that the proper number of MT attachments is formed. Inhibition of Hsp90 or Sgt1 dramatically reduces the levels of Mis12 subunits which

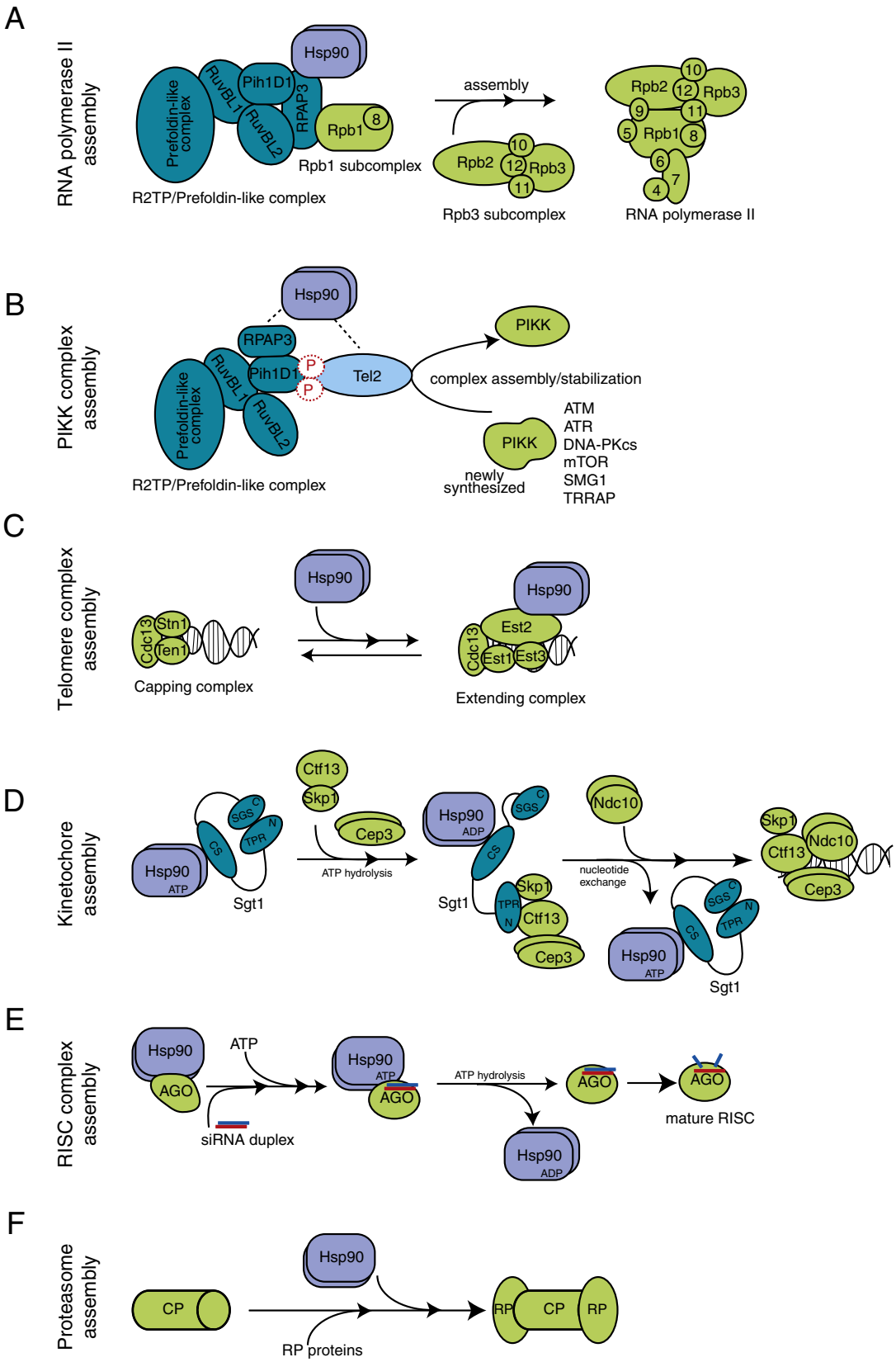

Fig. 3. The role of Hsp90 in the assembly of diverse protein complexes. Shown are simplified schematics of the role of Hsp90 in the assembly of RNA polymerase II, PIKK, telomere, kinetochore, RISC, and proteasome complexes. Refer to the text for details.

then leads to kinetochore defects resulting in delays in chromosome alignment due to inefficient formation of MT binding sites. Reminiscent of the yeast CBF3 complex, Mis12 complex turnover depends on SCF ubiquitin ligase subunit Skp1. These findings support a highly conserved role for Hsp90–Sgt1 in ensuring the fidelity of kinetochore complex assembly [92].

7. Role of Hsp90 in RNA induced silencing complex (RISC) assembly

Small RNAs, such as microRNA (miRNA) and small interfering RNA (siRNA) control diverse biological processes by RNA-mediated repression of gene expression, also known as RNA silencing [93,94]. The general molecular mechanism of RNA silencing involves the generation of siRNAs or microRNAs duplexes by a multidomain ribonuclease III enzyme, termed Dicer, from longer double-stranded RNA (dsRNA) precursors [95]. Various studies demonstrated that these small RNA duplexes are then loaded onto effector complexes such as RNA-induced silencing complexes (RISC), where they are bound by an Argonaute (AGO) protein [96–99]. Within the RISC complex, the small RNA duplexes are separated and one strand, termed the passenger strand, is destroyed [100], while the remaining guide strand is associated with the AGO protein and becomes able to inactivate complementary target mRNAs by endonucleolytic cleavage or translational repression [101].

Early work in this field suggested that Hsp90 might be involved in the assembly of functional RISC complexes in human cells [102,103] and plants [104]. More recently, several groups demonstrated that ATP-dependent loading of RISC with small RNA duplexes requires Hsp90/Hsp70 chaperones [5,6,105] (Fig. 3E). Using cell-free RISC assembly systems, these researchers showed that plant and animal AGO proteins interact with Hsp90. Blocking the ATPase activity of Hsp90 using geldanamycin and its derivatives significantly reduced RISC activity suggesting that Hsp90 activity is required for RISC assembly. Further experiments indicated that the specific function of Hsp90 in animal and plant RISC assembly might be different. In animals, Hsp90 together with Hsp70 is required for the ATP-dependent loading of the small RNA duplexes into RISC. In plants, RISC loading with duplex siRNA in the presence of Hsp90 can occur upon addition of ATPγS suggesting that ATP hydrolysis by Hsp90 is not required at this step. Instead, ATP hydrolysis is required for the dissociation of Hsp90 from AGO and this also induces conformational changes in AGO that facilitate the release of the passenger strand [5,6].

In summary, the Hsp90/Hsp70 chaperone machinery is required for the loading of small RNA duplexes into AGO proteins during the assembly of RISC complexes (Fig. 3E).

8. Role of Hsp90 in 26S proteasome assembly

Heat shock proteins closely cooperate with ubiquitin-proteasome machinery to control protein homeostasis. Most cellular proteins in eukaryotic cells are targeted for degradation by the 26S proteasome, usually after they have been covalently attached to ubiquitin in the form of polyubiquitin chain, functioning as a degradation signal [106]. The 26S proteasome is a ~2 MDa complex composed of a 20S catalytic core particle (CP) and bound at one or both ends by a 19S cap also known as the regulatory particle (RP) [107].

It was observed that the in vivo inactivation of Hsp90 using temperature sensitive *hsp82-4Δhsc82* mutant cells or by geldanamycin treatment causes disassembly of the 26S proteasome into the 20S core particle and lid complex components, indicating that Hsp90 is involved in maintaining proteasome structural integrity [108,109]. It was also found that Hsp90 tightly associates with the RP particle of 26S proteasome that would otherwise become structurally unstable and, hence, requires Hsp90 for assembly and maintenance (Fig. 3F). In contrast, 20S core particle does not require Hsp90. Additional experiments revealed that the ATPase activity of Hsp90 is needed for 26S proteasome assembly both in vivo and in vitro. The data suggest that the 26S proteasome is an Hsp90 client substrate and that Hsp90 is required for the maintenance of 26S proteasome complex assembly. Consistent with this, our global proteomic data on the yeast chaperone interactors [36] revealed that the RP complex has the third highest number of chaperone interactors highlighting a close cooperation between the folding and degradation machineries.

Although Hsp90 is involved in the assembly and maintenance of the proteasome, it is well established that inhibition of Hsp90 results in proteasomal degradation of target substrates. There is no clear answer at this stage to these seemingly contradictory observations, however, Hsp90 is probably not the sole protein that facilitates assembly of the proteasome. In this regard, down-regulation of the 26S proteasome due to geldanamycin addition was found to be only transient [109] indicating that other chaperones/proteins must play critical roles in the reassembly of the proteasome.

9. Concluding remarks

Over the past five years investigators have uncovered new links between Hsp90 and numerous cellular pathways and multiprotein complexes with the data highlighting the complexity of the Hsp90 chaperone system. The primary function of Hsp90 is thought to be protein stabilization, however, as described in this review, Hsp90 also has critical roles in the assembly of many vital multiprotein complexes. The diversity of the complexes affected by Hsp90 shows how this chaperone exerts a critical influence on a wide range of unrelated cellular pathways. The exact role of Hsp90 in these complexes varies. For some complexes, it is observed that Hsp90 mediates complex assembly by stabilizing an unstable protein subunit and facilitating its incorporation into the complex, while, in other instances, Hsp90 promotes the change in the composition of a given complex. The Hsp90 ATP hydrolyzing activity is generally required for all these functions. So far, the cochaperones Tah1/RPAP3 and Sgt1 have been identified to assist Hsp90 in its 'complex assembly' activities; it is likely that other cochaperones might be identified in the future that promote such an activity of Hsp90. Finally, we propose that promoting and maintaining complex assembly could be the major function of many other chaperones in the cell.

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