REVIEWS

HSP90 at the hub of protein homeostasis: emerging mechanistic insights

*Mikko Taipale**§, Daniel F. Jarosz**‡§ and Susan Lindquist**‡

Abstract | Heat shock protein 90 (HSP90) is a highly conserved molecular chaperone that facilitates the maturation of a wide range of proteins (known as clients). Clients are enriched in signal transducers, including kinases and transcription factors. Therefore, HSP90 regulates diverse cellular functions and exerts marked effects on normal biology, disease and evolutionary processes. Recent structural and functional analyses have provided new insights on the transcriptional and biochemical regulation of HSP90 and the structural dynamics it uses to act on a diverse client repertoire. Comprehensive understanding of how HSP90 functions promises not only to provide new avenues for therapeutic intervention, but to shed light on fundamental biological questions.

Macromolecular crowding
The exclusion of the volume available for biochemical reactions in solutions with high concentrations of macromolecules. Crowding promotes intermolecular interactions.

Misfolding
The adoption of an inappropriate conformation or unfolded state and/or aggregation that can occur as a result of macromolecular crowding, environmental stress or mutation.

**Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA.*
‡Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, USA.
§These authors contributed equally to this work.
Correspondence to S.L.
e-mail: sll@wi.mit.edu
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How proteins adopt the complex and dynamic structures they require for function is one of the most fundamental questions in biology. Although amino acid sequence and the laws of thermodynamics determine a protein's native conformation, the crowded cellular environment markedly influences folding of nascent polypeptide chains. The total protein concentration in the cytosol of a typical mammalian cell is ~300 mg per ml[1]. As a result of this macromolecular crowding, newly synthesized polypeptides and previously folded proteins with low stability expose hydrophobic surfaces, constantly risking misfolding and aggregation[2]. Both misfolding and aggregation pose a substantial burden to the organism, and protein folding defects can give rise to numerous human diseases[3].

Because abnormal protein folding can lead to many problems in a cell, all organisms have dedicated protein assemblies that maintain proteostasis and mitigate the life-threatening effects of heat and other stresses on the proteome. At the core of this repertoire are molecular chaperones and protein remodelling factors. These proteins help newly synthesized proteins to adopt their biologically active conformations[4], assemble and disassemble macromolecular complexes[1], mediate refolding of misfolded proteins and break up protein aggregates[5] (BOX 1). They also cooperate with the ubiquitin–proteasome system, targeting terminally misfolded proteins for degradation[6], and with translocation machineries to get proteins to their proper locations[7].

Together, these protein folding agents constitute a large, diverse and structurally unrelated group. Many are upregulated in response to heat and are therefore termed heat shock proteins (HSPs). HSP90 is one of the most conserved HSPs, present from bacteria to mammals, and is an essential component of the protective heat shock response. The role of HSP90, however, extends well beyond stress tolerance. Even in non-stressed cells, HSP90 is highly abundant and associates with a wide array of proteins (known as clients) that depend on its chaperoning function to acquire their active conformations.

HSP90 uses the energy generated by ATP binding and hydrolysis to fold proteins involved in signal transduction, protein trafficking, receptor maturation (that is, the attainment of an active conformation and intracellular nature) and innate and adaptive immunity. In doing so, HSP90 interacts with more than 20 co-chaperones, which guide its recognition of client proteins and modulate its biochemical activities. These activities are closely coupled to environmental perturbations. Specifically, under normal conditions there are high levels of HSP90 and thus an abundant chaperone reservoir, which buffers proteostasis against environmental stress. Under more extreme environmental conditions, the chaperone reservoir can be rapidly exhausted. Associated changes in the function of HSP90 clients can then exert marked effects on the relationship between genotype and phenotype, influencing human health, disease and evolutionary processes.

Box 1 | **How do chaperones promote protein folding?**

Although protein folding follows the same physical principles *in vivo* and *in vitro*, the crowded intracellular milieu poses additional challenges. Unstructured peptide backbones or hydrophobic amino acid residues exposed in nascent polypeptide chains, folding intermediates or partially unfolded proteins can engage in non-native intermolecular interactions. Such contacts compete with productive folding and promote protein aggregation. Chaperones prevent these off-pathway reactions by temporarily binding to partially folded intermediates and subsequently releasing them[4]. This chaperone cycle is usually driven by ATP hydrolysis, but the structural details differ greatly between chaperone families. Oligomeric chaperonins GroEL (also known as Cpn60) and the TCP1 ring complex (TRiC; also known as CCT) form large double ring-shaped folding cages that bind whole proteins and protein domains in molten globule conformations and encapsulate them to prevent non-productive intermolecular interactions during folding[159]. Heat shock protein 70 (HSP70) chaperones, by contrast, are monomeric, collaborate with HSP40 family co-chaperones to find their substrates and bind short linear stretches of hydrophobic residues[160]. Small HSPs form oligomeric complexes that bind denatured proteins on their surface in an ATP-independent manner for other chaperones to act on[161].

In addition to generalist chaperones, many chaperones are involved in the highly specific folding of individual proteins or the assembly of multiprotein complexes. Indeed, the first molecularly characterized chaperone, nucleoplasmin, specifically promotes the assembly of histones into nucleosomes[162]. Similarly, at least four chaperone proteins are required for the assembly of the proteasome[163]. Furthermore, chaperone function can be provided *in cis*. For example, bacterial α-lytic protease has a precursor domain, the function of which is to overcome a kinetic barrier in folding. Without the precursor domain, the protein is trapped in an intermediate conformation that is more stable than the biologically active, native structure[164].

Although the exact number of different chaperones in the cell is unknown, it is clear that a substantial fraction of the total cellular protein levels is dedicated to the maintenance of protein homeostasis.

In contrast to several other molecular chaperones, comprehensive mechanistic understanding of HSP90 functions remains elusive. The problem is challenging owing to the dynamic and transient nature of the complexes that HSP90 forms, the highly variable participation of co-chaperones and the low expression and unstable character of HSP90 clients. Recent biochemical and structural studies using new resources and technologies indicate that we are on the brink of achieving a new level of understanding of HSP90 function. In this Review, we discuss current findings on the HSP90 chaperone cycle and its regulation by co-chaperones, post-translational modifications and changes in the environment[8–14]. We also highlight new data on HSP90 clients and the diverse cellular processes they regulate[15–19]. Finally, we frame some remaining questions.

Cellular localization

HSP90 is a large dimeric protein found in almost every compartment of eukaryotic cells. Indeed, most eukaryotic genomes encode multiple compartment-specific HSP90 proteins, which arose early in evolution (BOX 2). Although structurally similar to cytosolic HSP90, they have distinct cellular functions, and probably also play important parts in health and disease. Because most data concern cytoplasmic HSP90, we focus on this HSP in this Review.

HSP90 is one of the most abundant proteins in the cytoplasm, where it constitutes 1–2% of total protein levels[20]. Some HSP90 translocates to the nucleus in response to stress and other stimuli[21–24]. HSP90 does not have a nuclear localization sequence, so this may occur by co-transport with client proteins. Cytosolic HSP90 can also be transported to other parts of the cell. For example, it has been detected in the extracellular matrix[25], where it matures metalloproteinase 2, mediating tumour cell invasiveness[26]. Furthermore, it can translocate to mitochondria, which have their own version of HSP90 but in some cases, such as malignant transformation, can acquire cytoplasmic HSP90 (REF. 27). Interestingly, inhibition of HSP90 function specifically in mitochondria induces selective apoptosis in tumour cells, and this could potentiallly be used for therapeutic interventions[27].

Transcriptional regulation

Despite its high basal abundance, HSP90 is further transcriptionally induced in response to stress. In bacteria, this induction is driven by the RNA polymerase subunit σ^{32} (encoded by *rpoH*)[28,29], which interacts with the RNA polymerase core to direct heat-inducible transcription of many genes. In *Burkholderia* spp. σ^{32} expression seems to be itself regulated in response to environmental stress by the products of the *rpoE* operon[30], adding an extra layer to the regulatory network.

In most eukaryotes, HSP90 expression approximately doubles in response to environmental stress. Frequently, eukaryotic genomes contain genes encoding constitutively expressed and inducible cytosolic HSP90. Inducible transcription is controlled by the transcription factor heat shock factor 1 (HSF1), which induces hundreds of genes in response to environmental stress[31,32]. Notably, under normal conditions HSF1 is a client of HSP90 but is then held in an inactive complex with HSP90 and HSP70. This interaction is broken under stress, releasing HSF1 for transcriptional activation. Thus, HSP90 also has an important role in regulating its own transcription[33].

Other transcription factors also influence eukaryotic HSP90 expression. For example, in human immune responses[34–36] the transcription of cytosolic HSP90α is induced by the binding of the nuclear factor-κB subunit p65 to consensus sequences in the HSP90 promoter[34], by NF-IL6β (also known as C/EBPδ) and signal transducer and activator of transcription 3 (STAT3) following interleukin-6 stimulation[35,36], and by STAT1 following interferon-γ stimulation. These mechanisms interface with HSF1 in diverse ways: STAT1 interacts with HSF1 synergistically, and STAT3 and HSF1 antagonize each other's activities[37].

Structure and conformational dynamics

The high intrinsic flexibility of structural conformation has hampered structural analysis of HSP90 for a long time. However, recent studies have uncovered structures of full-length HSP90 from bacteria[38], yeast[39] and mammals[40]. Their domain architecture is similar, but they exhibit large subdomain rotations relative to each other (discussed below). Several partial structures of HSP90 domains in complex with nucleotides, inhibitors and co-chaperones are also available. In conjunction with biochemical and structural dynamics data, these 'snapshots' have helped us begin to understand the complex conformational rearrangements underlying HSP90 function.

Proteostasis
The homeostasis of a functional protein in the cell, which is a product of its concentration, conformation, interactions, localization and turnover.

Chaperone
A protein that assists in the folding of a protein or assembly of a complex but does not otherwise contribute to the final structure or function of the product.

Protein remodelling factor
A protein that remodels the structure and conformation of its target macromolecules, often in a manner that requires ATP hydrolysis.

Ubiquitin–proteasome system
A major mechanism of protein degradation in a cell. The covalent tagging of proteins with another small protein, ubiquitin, targets them for degradation by macromolecular assembly of the proteasome.

Heat shock protein
(HSP). A protein induced in response to increased temperature, classified according to its size. Many HSPs function as molecular chaperones.

Co-chaperone
A protein that associates with and promotes the function of chaperones by modulating their chaperoning activity and/or regulating their substrate specificity. The definition of a co-chaperone is somewhat arbitrary, as some co-chaperones have chaperoning activity even when they are not associated with the core chaperones.

HSP90 structure. HSP90 shares structural homology with other ATPases in the GHKL (gyrase, HSP90, histidine kinase and MutL) superfamily[41]. Each monomer in the HSP90 dimer has a highly conserved amino-terminal domain (NTD) connected to a middle domain[41], followed by a carboxy-terminal domain (CTD) (FIG. 1). HSP90 binds ATP in its NTD, hydrolysing it following the interaction with clients and certain

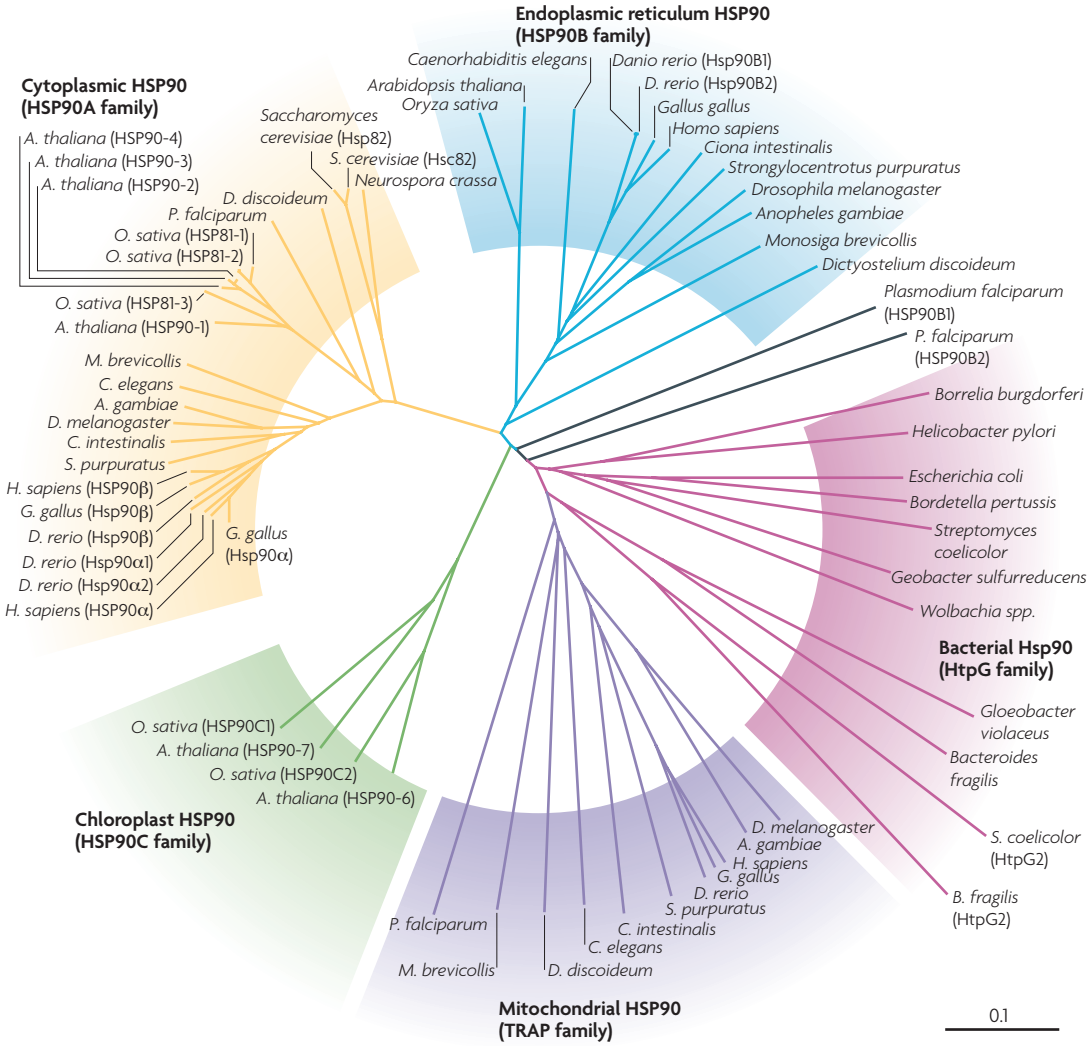
Box 2 | HSP90 family proteins

The heat shock protein 90 (HSP90) family is highly conserved. With the exception of archaea, organisms in all kingdoms of life have one or more genes encoding HSP90. The HSP90 family can be divided into five subfamilies: cytosolic HSP90A, endoplasmic reticulum (ER)-localized HSP90B, chloroplast HSP90C, mitochondrial TNFR-associated protein (TRAP) and bacterial high temperature protein G (HtpG)[165] (see the figure; scale bar represents genetic distance). HtpG is found in most bacteria but, in contrast to its eukaryotic counterparts, it is not an essential gene in non-stressful conditions and has only modest effects on growth at high temperatures.

The four eukaryotic HSP90 subfamilies evolved from an HtpG-like group[165]. The TRAP family of mitochondrial HSP90s is most closely related to the bacterial HtpG group; however, it is unlikely to be of endosymbiotic origin[165]. Instead, it seems to have evolved independently of the HSP90A, HSP90B and HSP90C families during early eukaryote evolution. The function of TRAP is poorly understood, but human TRAP has been shown to protect cells from oxidative stress-induced apoptosis[166].

The ER-localized HSP90B proteins, known as GRP94 in humans, have a role in ER protein quality control. All known GRP94 clients are in secretory pathways or are found on the cell surface[167]. GRP94 is strongly induced by perturbations in ER protein homeostasis, such as starvation for glucose or exposure to tunicamycin, which inhibits N-linked glycosylation of proteins. Interestingly, it seems that the cytosolic HSP90A and chloroplast HSP90C families evolved from the ER-localized HSP90B group[165].

The HSP90A group is the largest, most widespread and best-studied of the HSP90 families. Evolutionarily recent duplication events have resulted in multiple genes encoding cytosolic HSP90 in many organisms. For example, most vertebrates have two genes encoding HSP90: *HSP90AA/1* and *HSP90AB/1*. The function of these paralogues is highly overlapping, although a few paralogue-specific functions have been reported[26].



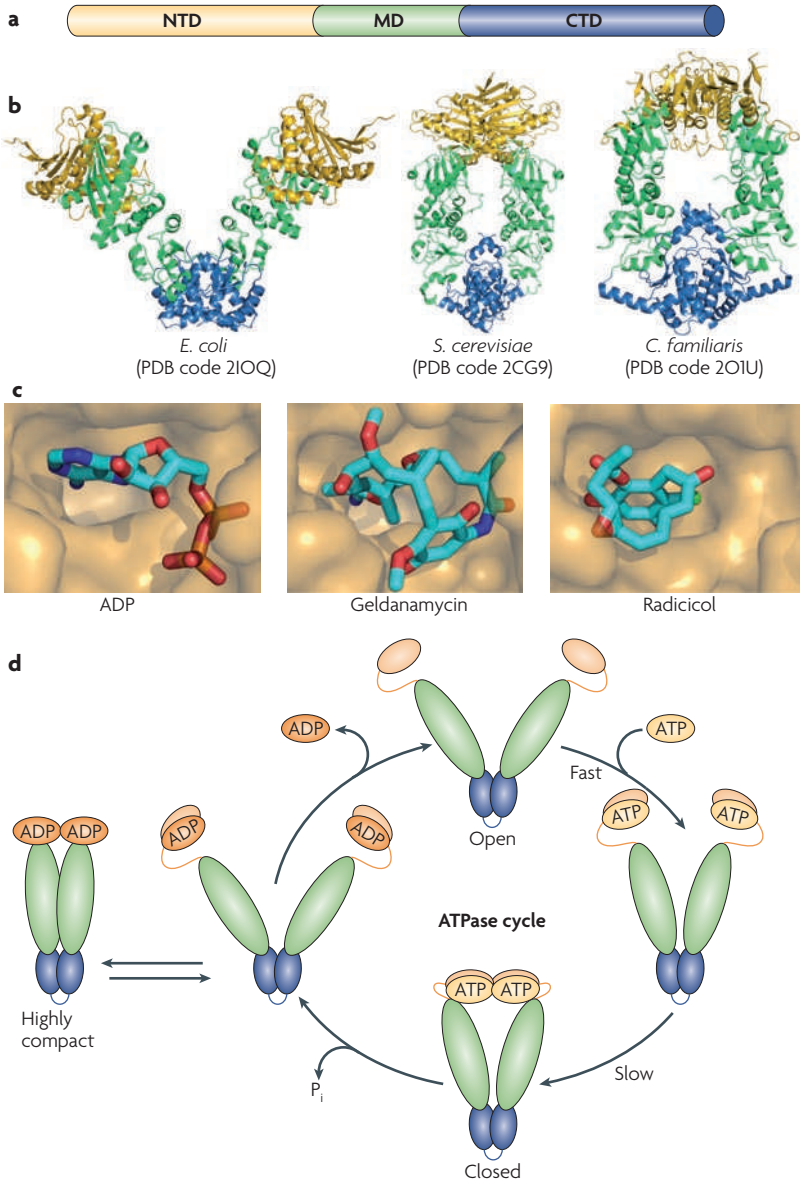


Figure 1 | **HSP90 domain structure and chaperone cycle. a** | Domain structure of heat shock protein 90 (HSP90), consisting of an amino-terminal domain (NTD), middle domain (MD) and carboxy-terminal domain (CTD). **b** | Structures of full-length Hsp90 dimers from *Escherichia coli*, *Saccharomyces cerevisiae* (both cytosolic) and *Canis familiaris* (GRP94; an endoplasmic reticulum HSP90). Colours indicate domains from part **a**. **c** | Binding of nucleotide and HSP90 inhibitors to the NTD nucleotide-binding pocket. Both nucleotide (ADP) and the natural product inhibitor geldanamycin bind towards the front of the pocket, whereas the structurally distinct natural product inhibitor radicicol binds at the back of the pocket. **d** | Schematic of the proposed HSP90 conformational cycle and the role of nucleotide hydrolysis. ATP binding leads to a closed HSP90 conformation. Hydrolysis of ATP to ADP leads to a second state that is structurally unclear. ADP dissociation restores HSP90 to its open conformation. PDB, protein data bank.

X-ray crystallography
A method for determining the precise arrangement of atoms in a crystal, based on its diffraction pattern in an X-ray beam. This technique can be applied to protein crystals to obtain extremely high-resolution structures.

co-chaperones. The ATP-binding site (FIG. 1c) consists of an α- and β-sandwich motif[42–44]. Structurally unrelated natural product inhibitors of HSP90 displace ATP and block HSP90 function with high specificity[45]. The strong conservation of the ATP-binding pocket allows these inhibitors to serve as extremely useful tools for probing HSP90 function from plants to animals to fungi. Several conserved amino acid residues in the NTD comprise a molecular 'lid' that closes over the nucleotide-binding pocket in its ATP-bound, but not ADP-bound, state. Efficient ATP hydrolysis also requires a catalytic Arg residue from a loop in the middle domain to dock with the nucleotide-binding pocket[46]. Furthermore, in the HSP90 dimer, a stabilizing interaction with the N terminus of the opposite monomer promotes activity[47].

The NTD is connected to the HSP90 middle domain by a charged linker, which has an important role in HSP90 function[9,48]. Indeed, mutations in this region impair client activation and prevent regulation by certain co-chaperones[9]. The middle domain itself consists of two αβα motifs connected by a series of α-helices[46] and is thought to have an important role in client recognition. Structural and mutagenesis studies[38,46,49,50] revealed a hydrophobic patch and amphipathic protrusion in the middle domain that are crucial for client–HSP90 interactions.

The CTD of HSP90 mediates dimerization[50,51] and is less conserved in sequence than the rest of the protein. Structurally, it is a mixed α- and β-domain. Two α-helices from each monomer form a four-helix bundle at the dimer interface[38,41]. Although a low-affinity nucleotide-binding site[52,53] has not been confirmed, the five C-terminal residues (Met-Glu-Glu-Val-Asp; the MEEVD motif) make up a highly conserved tetratricopeptide repeat (TPR) domain-binding site, which mediates interaction with many co-chaperones (see below)[54].

Dynamic chaperone cycle. Large and highly dynamic conformational shifts are crucial for HSP90's ability to recognize its diverse client repertoire. Recent work is beginning to elucidate these transitions and explain the role of ATP hydrolysis in facilitating them. Early biochemical studies indicated that ATP binding facilitates the transition from an open (ADP-bound) to a closed (ATP-bound) form[55–57]. Later structural studies and mutagenesis suggested that ATP hydrolysis is coupled to additional structural rearrangements[58–60]. These rearrangements, termed the HSP90 chaperone or ATPase cycle, are essential for client maturation. The weak intrinsic ATPase activity of HSP90 is affected by client binding and its interaction with certain co-chaperones[33,61], as discussed below.

Despite overall structural similarity between HSP90s, X-ray crystallography has revealed surprising differences in domain arrangements between species (FIG. 1b). These differences might arise from crystal packing artefacts or differences between species in the chaperone cycle. A more interesting possibility is that they are snapshots of rearrangements that normally occur transiently during the course of a conserved chaperone cycle. Recently, electron microscopy (EM) allowed the direct observation of individual HSP90 molecules from *Escherichia coli*, *Saccharomyces cerevisiae* and *Homo sapiens*[62]. Unexpectedly, in contrast to other chaperones (and indeed most nucleotide-binding proteins), binding of a nucleotide does not lock HSP90 into a specific conformation. Instead, it shifts a pre-existing equilibrium among several conformational states in favour of a more closed and presumably active

conformation. This high degree of conformational flexibility could certainly be regulated in cells by the interaction with clients and co-chaperones[63–65], many of which vary between species. In their absence, HSP90's conformational equilibrium can be affected by environmental factors, such as pH[14].

Without nucleotide binding, both the open and closed conformations can be found in *E. coli* and *S. cerevisiae*, whereas almost all *H. sapiens* HSP90s are in an open conformation. Treatment of the *E. coli* and *S. cerevisiae* proteins with the non-hydrolysable ATP analogue AMPPNP drives them towards a more closed conformation. This effect is detected only in *H. sapiens* HSP90 by trapping with chemical cross linkers. Negative-stain EM images have revealed an ADP-bound conformation of HSP90 that is even more compact than the closed, ATP-bound state[62]. This finding is in agreement with previous structural investigations[38], but in contrast to some biochemical studies[55–57]. Recent analysis using hydrogen–deuterium (H–D) exchange and fluorescence[63–66], which provides structural information on rapid timescales under physiological conditions, have confirmed most nucleotide-induced states observed to date, but suggest that the most compact ADP conformation is not common at physiological protein concentrations.

Together, these studies give rise to a kinetic model of how ATP hydrolysis drives conformational changes in the HSP90 chaperone cycle[63,66] and how these changes can be modulated by co-chaperones[63–65]. ATP binding causes a molecular lid to close over the nucleotide-binding site, followed by a slow transition to a closed (also referred to as tense) state, in which the NTDs of each monomer interact. Hydrolysis of ATP leads to a second, still structurally ambiguous, state. ADP dissociation restores HSP90 to its original open conformation (FIG. 1d). Importantly, these large structural changes are the rate limiting steps of the ATPase cycle and occur much slower than ATP hydrolysis itself[63,66].

Regulation of HSP90 function

HSP90 is subject to a remarkable degree of biochemical regulation. In addition to ATP binding and hydrolysis, interaction with diverse co-chaperones directs the chaperone cycle. Both HSP90 and co-chaperones are further regulated by post-translational modifications. The combinatorial effects of these factors are just beginning to be understood.

Regulation by co-chaperones. In eukaryotic cells, more than 20 co-chaperones have been identified (FIG. 2). In many cases their biological roles remain largely unknown. Furthermore, some co-chaperones (for example, Cdc37) are crucial in certain organisms but apparently absent in others[67]. Those that have been studied are known to modulate HSP90 function in four ways: they coordinate the interplay between HSP90 and other chaperone systems, such as HSP70; they stimulate or inhibit the ATPase activity of HSP90; they recruit specific classes of clients to HSP90; and, through their enzymatic activities, they contribute to various aspects of the chaperone cycle.

The largest subset of co-chaperones are the ones containing TPR domains (consisting of helical hairpins), which bind to the MEEVD motif in the HSP90 CTD[54]. This class of co-chaperones is remarkably diverse, despite having a shared mechanism of HSP90 binding. Some recruit specific proteins to HSP90 (for example, WAF1/CIP1 stabilizing protein 39 (WISp39; also known as FKBPL)[68]) or other multiprotein complexes (for example, translocase of outer membrane 70 kDa (TOM70)[7] and HSC70 and HSP90-organizing protein (HOP; also known as p60 and STI1)[69]). Others have accessory functions, ranging from phosphatase activity (such as Ser/Thr protein phosphatase 5 (PP5)[70]) to prolyl isomerase function (such as the immunophilins cyclophilin 40 (CYP40; also known as PPID) and FK506-binding proteins (FKBPs)[69,71]). These functions seem to be dispensable for client activation in at least one case (that of FKBP52-dependent potentiation of glucocorticoid receptor function[72–74]), but their importance is only now beginning to be addressed.

One particularly important role of TPR domain-containing co-chaperones is to facilitate the cooperative, successive action of HSP40, HSP70 and HSP90 on client proteins to achieve client maturation. The best-understood example is HOP (FIG. 2a). Its multiple TPR domains mediate simultaneous binding to both HSP90 and HSP70 (some HSP70s in the eukaryotic cytosol and nucleus contain a TPR domain-binding motif (Ile-Glu-Glu-Val-Asp), so they can also bind co-chaperones with TPR domains), facilitating a dynamic cycle in which clients are matured by the successive action of each chaperone. For example, the progesterone receptor, perhaps the best-characterized HSP90 client, first binds HSP40, which recruits ATP-bound HSP70 (REF. 75). This association triggers ATP hydrolysis in HSP70 and tight binding between HSP70–ADP and the progesterone receptor. HSP90–HOP then binds HSP70–ADP, allowing delivery of the progesterone receptor to HSP90. The final maturation step includes binding of p23 (also known as PTGES3 and as Sba1 in yeast) and ATP, which leads to the dissociation of HOP and HSP70 (REF. 75).

Many co-chaperones either inhibit HSP90's ATPase activity (for example, HOP, Cdc37 and p23)[76–78] or enhance it (for example, activator of HSP90 ATPase homologue 1 (AHA1) and Cpr6)[78,79], probably reflecting the different stages of the chaperone cycle at which they act. For example, AHA1 accelerates the early conformational transitions of the ATPase cycle, whereas HOP inhibits them[63]. In general, the co-chaperones that inhibit ATPase activity are more likely to be involved in client loading or the formation of mature HSP90 complexes, whereas those that enhance the activity are more likely to be activators of the HSP90 conformational cycle. Of more than 20 known co-chaperones, the structural basis of their effect on HSP90's ATPase activity is beginning to be understood in only four cases: AHA1, Cdc37, p23 and SGT1.

AHA1 is one of the few co-chaperones that is known to enhance HSP90's ATPase activity. A co-crystal structure of the middle domain of HSP90 with the N terminus of AHA1 (REF. 80) shows that AHA1 facilitates contacts between

Electron microscopy
(EM). A form of microscopy that uses a particle beam of electrons to obtain high magnifications (up to 1,000,000-fold). Although EM generally provides lower resolution than other structural techniques, molecules can usually be visualized under closer to physiological conditions.

Hydrogen–deuterium (H–D) exchange
A reaction in which hydrogen atoms, typically backbone amides in the context of a protein, exchange with deuterium in a D_2O-based buffer. The rate at which this exchange occurs is related to how solution-accessible each position is, and indicates whether a given residue is on the surface of the protein or more buried. Surface hydrogen atoms involved in hydrogen bonding interactions will not exchange.

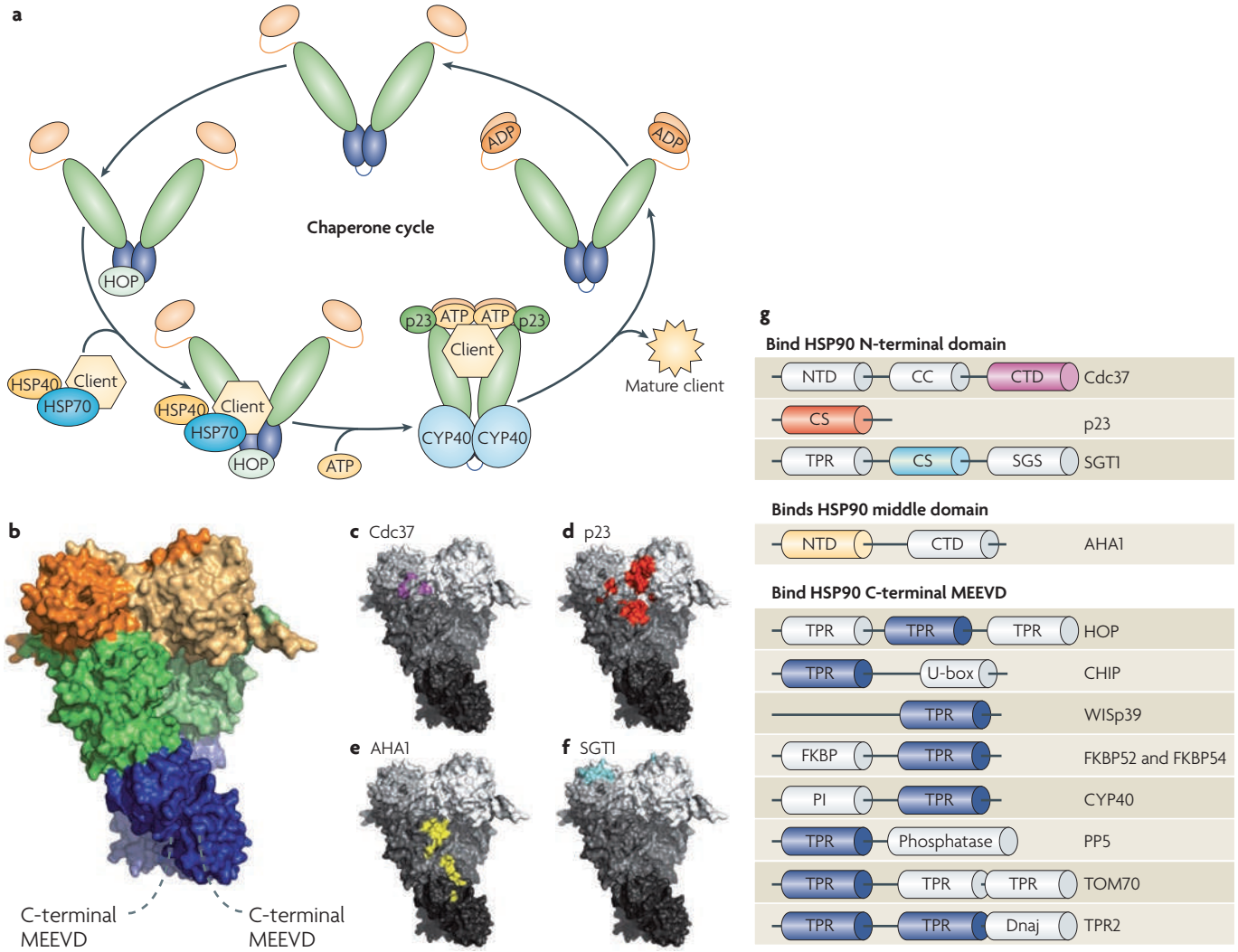


Figure 2 | **Co-chaperones facilitate client maturation by interacting with distinct surfaces on HSP90.** **a** | Schematic of the maturation of the progesterone receptor mediated by the cooperation between heat shock protein 90 (HSP90), HSP70 and co-chaperones. The progesterone receptor binds HSP40 and HSP70, which then binds the HSP90–HOP (HSC70 and HSP90-organizing protein; also known as p60 and STI1) complex, leading to delivery of the client. Finally, the co-chaperone p23 (also known as PTGES3 and as Sba1 in yeast) and ATP bind the complex, promoting client maturation and complex dissociation. The role of cyclophilin 40 (CYP40; also known as PPID) is not well understood but it regulates the activity of client proteins in the final stages of the chaperone cycle. **b** | The crystal structure of yeast Hsp90 in a closed conformation, bound to a non-hydrolysable ATP analogue (AMPPNP) and the co-chaperone Sba1. Co-chaperones can bind the Hsp90 amino-terminal domain (NTD; orange), middle domain (green) or the Met-Glu-Glu-Val-Asp (MEEVD) motif in the carboxy-terminal domain (CTD; blue). **c**–**f** | Cdc37 and p23, which both inhibit HSP90 ATPase activity, bind near the ATP-binding pocket of HSP90. The co-chaperone activator of HSP90 ATPase homologue 1 (AHA1) binds along the HSP90 middle domain and facilitates ATP hydrolysis by promoting middle domain contacts with the active site in the NTD. SGT1, which, like p23, binds HSP90 through its CHORD and Sgt1 (CS) domain, interacts with a distinct site on the HSP90 NTD. **g** | Domain organization of co-chaperones that have been shown to bind HSP90. Domains that interact with HSP90 are coloured as in parts **c**–**f**. CC, coiled coil; CHIP, C terminus of HSP70-interacting protein; FKBP, FK506-binding protein; PI, prolyl isomerase; PP5, protein phosphatase 5; SGS, SGT1 specific; TOM70, translocase of outer membrane 70 kDa; TPR, tetratricopeptide repeat; WISp39, WAF1/CIP1 stabilizing protein 39.

HSP90's middle domain catalytic loop and the NTD nucleotide-binding site. This stabilizes an HSP90 conformation that promotes efficient ATP hydrolysis (FIG. 2f). Indeed, recent studies suggest that AHA1 stimulates the ATPase activity of HSP90 through an asymmetric interaction that promotes a closed conformation in which the NTDs from each HSP90 monomer associate[81]. Interestingly, the ability of AHA1 to accelerate the rate limiting conformational changes that precede ATP hydrolysis can be seen even in the absence of bound nucleotide[63].

Two structures illustrate how co-chaperones inhibit HSP90 activity. Cdc37 links kinase clients to HSP90, interacting with the kinase through its N terminus and

HSP90 through its C terminus[82–85]. The structure of the complex suggests at least three ways in which Cdc37 inhibits HSP90's ATPase activity. First, Cdc37 interacts with residues that are crucial for ATPase activity (in the HSP90 NTD), thereby precluding access of the middle domain catalytic loop to the ATP-binding pocket. Second, Cdc37 blocks the ATP-binding pocket, holding HSP90's molecular lid in an open, inactive conformation. Third, Cdc37 precludes NTD dimerization in the closed state of the HSP90 ATPase cycle (FIG. 2c).

p23 associates with HSP90 late in the chaperone cycle, facilitating the maturation of client proteins[86,87]. A co-crystal structure of yeast p23 with full-length Hsp90 (REF. 39) reveals that p23 binds the Hsp90 NTD, reducing its conformational flexibility (FIG. 2d). For this association, Hsp90 must bind ATP, its molecular lid must be closed, its NTDs must associate with each other, and its middle domain catalytic loop must dock with the NTD ATP-binding pocket. This conformational state is normally achieved transiently before ATP hydrolysis and might seem to favour Hsp90 ATPase activity. However, p23 binding clearly abolishes ATPase activity while stimulating ATP binding[64], underscoring the importance of conformational flexibility in a functional chaperone cycle. Stabilization of a closed, normally transient conformation by p23 probably facilitates client binding and maturation[64].

Another p23-related co-chaperone, SGT1, binds different surfaces on HSP90, despite structural similarity with p23 (REF. 88) (FIG. 2e). SGT1 binds HSP90's NTD, but not its molecular lid. Perhaps as a result of this, SGT1 binding does not affect the HSP90 ATPase cycle.

Understanding these diverse co-chaperone functions provides insight on HSP90 chaperone machinery and also has potential therapeutic implications. In 2003, a high profile paper reported that many cancer cells have increased levels of co-chaperone-bound HSP90 (REF. 89). This would potentially explain the well-known sensitivity of tumour cells to HSP90 inhibitors. However, these results have been questioned by other studies[90]. In any case, the importance of co-chaperones in HSP90 function is clear from the effect of their depletion. As just one example relevant to human disease, the maturation defect of the cystic fibrosis-associated mutant protein CFTRdel508, an HSP90 client, can be reversed by depletion of the co-chaperone AHA1 (REF. 91).

Regulation by post-translational modifications. HSP90 function is also regulated by numerous post-translational modifications such as acetylation, phosphorylation and nitrosylation. Phosphorylation of HSP90β's charged linker regulates the interaction with the client aryl hydrocarbon receptor (AHR). Mutation of phosphorylated Ser225 and Ser254 to Ala in the HSP90 middle domain increases HSP90 binding to AHR, suggesting that phosphorylation negatively regulates complex formation[92]. Phosphorylation also inhibits client interaction more generally. Indeed, deletion of the yeast Hsp90 co-chaperone protein phosphatase 1 (Ppt1) causes hyperphosphorylation of Hsp90 and impedes the maturation of several client proteins[93].

In other cases, HSP90 phosphorylation facilitates client maturation. SRC-dependent phosphorylation of HSP90β (on Tyr300) increases the chaperone's association with the client endothelial nitric oxide synthase (eNOS) on activation of vascular endothelial growth factor signalling[94]. Similarly, phosphorylation of Tyr24 in yeast Hsp82 (Tyr38 in human HSP90α) by Swe1 kinase inhibits Hsp82's ATPase activity and dimerization, and leads to its polyubiquitylation in the cytoplasm. Moreover, Tyr24 phosphorylation markedly reduces the stability of many kinase clients and the activity of Hsf1 (REF. 95).

Mammalian HSP90 is also acetylated at many sites. Acetylation of Lys294 in the middle domain by an unknown acetylase inhibits both client protein maturation and co-chaperone binding[96,97]; histone deacetylase 6 deacetylates this residue *in vivo*. To date, only two papers report nitrosylation of HSP90, but both suggest an important regulatory role for this post-translational modification. Nitrosylation of Cys597 in the HSP90β CTD inhibits eNOS activation *in vivo*[8,98], and *in vitro* *S*-nitrosylation inhibits HSP90 ATPase activity[98] and shifts the conformational equilibrium of the chaperone cycle[8]**.** Other post-translational modifications have been reported for HSP90, but their functional importance is unknown.

In addition to HSP90, at least one of its co-chaperones is post-translationally regulated. In mammalian and yeast cells, Cdc37 is phosphorylated by casein kinase 2α (CK2α; also known as CSNK2A1) at Ser13 (Ser14 in yeast). The co-chaperone PP5 dephosphorylates Ser13, but only in the context of the HSP90–Cdc37–CK2α complex. Because mutations mimicking the non-phosphorylated (Ser13Ala) or phosphorylated (Ser13Glu) form both disrupt client protein function[12], it is likely that cyclical regulation of Ser13 phosphorylation is an essential part of the HSP90 chaperone cycle with kinase substrates.

HSP90 clients

Establishing that a protein is an HSP90 client relies on two lines of evidence. First, it must physically interact with HSP90. Second, inhibition of HSP90's function must result in a lower level of client protein activity. Most commonly, the latter leads to degradation in the proteasome[99], but aggregation can also occur.

A wide range of HSP90 clients. Early experiments identified two main classes of HSP90 clients: protein kinases and nuclear steroid receptors. Indeed, HSP90 was initially discovered during affinity purification of the first molecularly characterized oncogene, the protein Tyr kinase v-Src. A 90 kDa protein (then named pp90) co-precipitated with newly synthesized v-Src from Rous sarcoma virus-transformed chicken cells[100]. It also co-purified with other virally encoded Src-like Tyr kinases such as Fes, Fps and Yes[101]. At the same time, HSP90 was detected in several nuclear hormone receptor complexes[102–104]. Although the best-understood clients are steroid hormone receptors and protein kinases, many others have been discovered, such as eNOS[105],

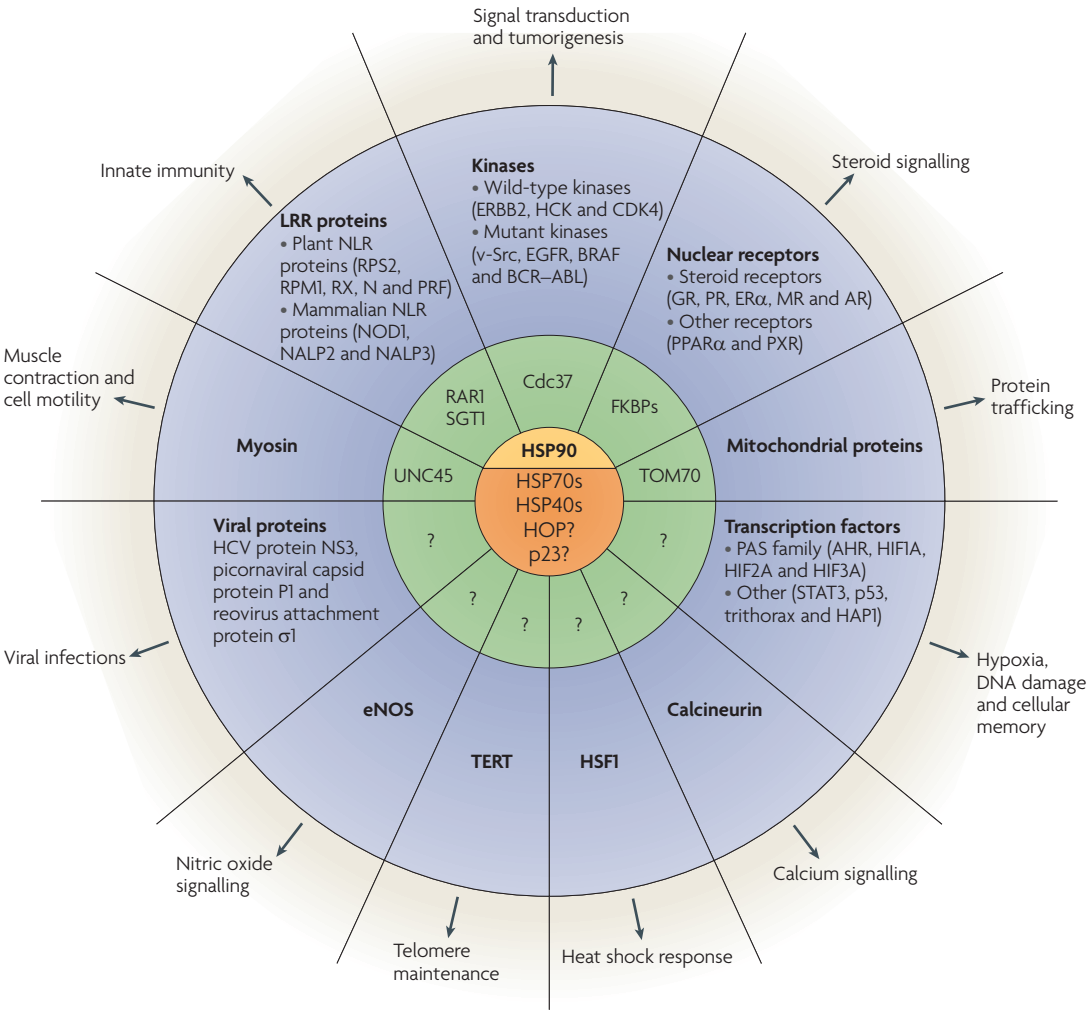


Figure 3 | **HSP90 regulates diverse cellular processes through its interaction with client proteins.** Heat shock protein 90 (HSP90) associates with core co-chaperones (such as p23 (also known as PTGES3 and as Sba1 in yeast), HSC70 and HSP90-organizing protein (HOP; also known as p60 and STI1) and the HSP40–HSP70 chaperone system; orange) and other co-chaperones (green). Adaptor co-chaperones such as Cdc37 and SGT1 associate with a limited number of proteins or protein domains (blue). However, for most HSP90 clients, it is not known which co-chaperones, if any, are associated in the complex (indicated by question marks). AHR, aryl hydrocarbon receptor; AR, androgen receptor; CDK4, cyclin-dependent kinase 4; EGFR, epidermal growth factor receptor; eNOS, endothelial nitric oxide synthase; ERα, estrogen receptor-α; FKBP, FK506-binding protein; GR, glucocorticoid receptor; HAP1, haeme activator protein 1; HCK, haemotopoietic cell kinase; HCV, hepatitis C virus; HIF, hypoxia-inducible factor; HSF1, heat shock factor 1; LRR, Leu-rich repeat; MR, mineralocorticoid receptor; N, nucleoprotein; NALP, NACHT, LRR and PYD domains-containing protein; NLR, nucleotide-binding LRR; NOD1, nucleotide-binding oligomerization domain-containing protein 1; PPARα, peroxisome proliferator-activated receptor-α; PR, progesterone receptor; PRF, *Pseudomonas* resistance and fenthion sensitivity; PXR, pregnane X receptor; RPS2, resistance to *Pseudomonas syringae* 2; STAT3, signal transducer and activator of transcription 3; TERT, telomerase reverse transcriptase; TOM70, translocase of outer membrane 70 kDa.

telomerase reverse transcriptase (TERT)[106], and transcription factors and chromatin proteins (such as PAS family transcription factors, p53, STAT3 and trithorax)[107–110] (FIG. 3). Collectively, these place HSP90 at numerous vital regulatory hubs.

In plants, HSP90 and the co-chaperone SGT1 associate with many NLR (nucleotide-binding, Leu-rich repeat (LRR)) proteins, which are cytoplasmic sensor proteins required for innate immunity[111,112]. Their LRR domains recognize pathogen effector molecules, which leads to the activation of downstream signalling. HSP90 and SGT1 are required for the stability of the sensor complex[111,112], so mutations in HSP90 or SGT1, or inhibition of HSP90, reduce the levels of NLR proteins and compromise the immune response against many pathogens[111,112]. Interestingly, mammalian NOD-like receptors, which are structurally related to plant NLRs, also functionally interact with HSP90 and SGT1 (REF. 113). As HSP90 has also been implicated in antigen processing[114,115], it seems it has a role in both innate and adaptive immunity in vertebrates.

HSP90 is also involved in protein trafficking and secretion by chaperoning components of the Rab GTPase cycle. HSP90 binds to and regulates the function of RhoGDIα (also known as ARHGDIA), a GDP dissociation inhibitor for Rab proteins[177]. RhoGDIα is, in turn, required for recycling of numerous Rab proteins that regulate membrane trafficking. RAB3A-dependent neurotransmitter release at the synapse and RAB1B-dependent endoplasmic reticulum (ER)-to-Golgi trafficking are HSP90-dependent processes and it is likely that HSP90 also regulates other Rab proteins. Pharmacological inhibition of HSP90 impairs both ER-to-Golgi and intra-Golgi trafficking. This is at least partly regulated by the TPR domain-containing co-chaperone TPR protein 1 (TTC1; also known as TPR1)[178]. Furthermore, HSP90 inhibition leads to impaired protein secretion in both mammalian and yeast cells, illustrating a conserved and global role in protein trafficking.

Recent studies have also implicated HSP90 in diverse aspects of RNA processing. Yeast Hsp90 and its co-chaperone TPR-containing protein associated with Hsp90 (Tah1) regulate the function of the R2TP (Rvb1, Rvb2, Tah1 and Pih1) complex by chaperoning the protein interacting with Hsp90 1 (Pih1) subunit[116]. The R2TP complex is required for the accumulation of C/D box small nuclear RNPs, and ribosomal RNA processing, Pih1 deletion or Hsp90 inhibition impairs these processes[116]. A homologous complex exists in mammalian cells and its function also requires HSP90 (REF. 117). HSP90 inhibition also causes the disappearance of P bodies — cytoplasmic messenger ribonucleoprotein (mRNP) foci that contain translationally repressed mRNAs and RNA processing factors[118]. The mechanistic link might be HSP90 chaperoning of Argonaute proteins, some of which localize to P bodies[119–121]. Argonaute proteins, in turn, are essential components of microRNA, short interfering RNA and piwi-associated RNA (piRNA) pathways, which mediate post-transcriptional silencing in eukaryotes. HSP90 also affects small RNA processing, but the mechanism by which it achieves this is still unclear at the biochemical level. However, the HSP90 chaperone machinery unequivocally has a role in small RNA pathways *in vivo*. In plants, the HSP90 co-chaperone SQUINT (a CYP40 orthologue) is required for microRNA activity[122]. In flies, compromise of HSP90 function leads to reduced piRNA accumulation and activation of numerous transposons, which are normally silenced by the piRNA pathway[123].

The above examples provide only a glimpse of the extraordinarily diverse clientele of HSP90, and, owing to space limitations, we cannot discuss them all. The Picard laboratory maintains a comprehensive and actively updated list of HSP90 clients in multicellular organisms. In recent years, however, knowledge of HSP90 clients has grown mainly owing to genome-wide studies in yeast, in which 1,232 unique proteins interact physically or genetically with Hsp90 (REF. 124) (see the BioGRID database). In other words, ~20% of yeast proteins are influenced by Hsp90 function, making it the most highly connected protein in the yeast genome. This is in stark contrast with the view that Hsp90 has a limited clientele compared with other classes of chaperones such as GroEL (also known as Cpn60) and GroES (also known as Cpn10), which mediate the folding of ~10% of proteins in *E. coli*[125].

How can we explain this apparent discrepancy? First, yeast can grow at ambient temperature with only 5% of wild-type HSP90 levels[20], suggesting that HSP90's chaperoning capacity vastly exceeds demand under normal conditions. This would seem incongruous with actively folding ~20% of the proteome. Second, HSP90 has only a limited effect on protein folding *in vivo*[126]. Third, rather than binding fully denatured polypeptide chains, as do HSP60 and HSP70, HSP90 binds partially folded intermediate conformations[41], suggesting that it matures a restricted set of proteins that occupy metastable conformations late in their folding pathways. Certainly this is the case for all of HSP90's well-characterized clients.

Most likely, the unexpectedly large number of potential clients identified in genome-wide screens reflects two features intrinsic to HSP90's biology. First, HSP90 clients occupy central positions in many biological networks (FIG. 3). Thus, disruption of their folding would cause pleiotropic effects on other proteins. Indeed, HSP90-interacting proteins themselves interact with more proteins than expected by chance[15]. Second, the methods used to date detect many proteins in addition to HSP90 clients. Genetic interactions capture entire biological processes that require HSP90 function. Physical interactions reveal co-chaperones, bona fide clients and other proteins that associate with HSP90, but do not require its chaperoning function for their maturation. This notion is supported by gene ontology analysis of genome-wide yeast data: proteins that physically interact with HSP90 are enriched in co-chaperones and other protein-folding factors, whereas proteins involved in intracellular transport and protein localization are over-represented in genetic interactors (M.T. and S.L., unpublished observations).

These findings highlight the complexities intrinsic to the analysis of HSP90–client interactions on a genome-wide scale. Because association with client proteins is often transient, interaction can be difficult to observe on the timescale of most techniques. Genetic analysis is also confounding because most clients, in addition to HSP90 itself, are highly connected to multiple regulatory networks.

In reality, proteins may not fall naturally into two classes — clients and non-clients. It is more likely that the degree of HSP90 binding varies continuously, as is the case for GroEL in bacteria[125]. Thus, the transition between HSP90 client and non-client may not always be clear-cut. Furthermore, whether an individual protein is an HSP90 client could well be influenced by extrinsic factors. Temperature undoubtedly has a great effect, but so could cell and tissue type, interactions with other proteins, alternative splicing, post-translational modifications and cell signalling events. For example, interaction between eNOS and HSP90 is enhanced on eNOS activation by various signals[105]. Steroid hormone receptors also disassociate from HSP90 after binding their ligands[127]. Such context-dependent interactions have also been reported for kinases[128], suggesting that a large

Small nuclear RNP
A low molecular weight RNA, associated with proteins. Small nuclear RNPs mediate the splicing of primary RNA transcripts.

Argonaute
A family of proteins that are characterized by the presence of two homology domains: PAZ and PIWI. These proteins are essential for diverse RNA silencing pathways.

portion of the HSP90 clientele can change in response to environmental signals[15]. The great strength of the recent high-throughput studies is that they identify candidates on an unprecedented scale and in an unbiased way. The application of other approaches to these candidates and the enormous importance of the task to human biology and disease research suggest that a detailed and quantitative assessment of the HSP90 client profile in humans will soon be in our grasp.

How does HSP90 recognize its clients? Despite a long list of HSP90 clients, we know surprisingly little about the molecular basis of client recognition. This stands in striking contrast to many other chaperones (BOX 1). For example, HSP70 recognizes short hydrophobic motifs that are exposed in nascent polypeptide chains or partially denatured proteins[129], and the clients of the TCP1 ring complex (TRiC; also known as CCT) in the cytosol are proteins with β-strands that tend to stay in a molten globule conformation during maturation[130]. Despite intense investigation, common sequences or structural motifs among HSP90 clients have remained elusive. The diversity of proteins that rely on HSP90 chaperoning function suggests that a recognition motif per *se* does not exist. Instead, co-chaperones and other accessory factors play an important part in client recognition, as exemplified by the kinase-specific co-chaperones Cdc37 and SGT1, which seem to be specific to LRR-containing proteins.

A series of experiments illustrates why it has been difficult to identify the HSP90-binding motif. One study compared HSP90 binding to the closely related receptor protein Tyr kinases epidermal growth factor receptor (EGFR) and ERBB2 (REF. 99). EGFR associates with HSP90 only transiently during its synthesis, whereas ERBB2 and HSP90 form a stable complex. Surface charge on the αC–β4 loop of the kinase domain was identified as an important determinant of HSP90 binding. A single mutation (Asp770Gly) in EGFR strengthened the interaction with HSP90, and the reciprocal change in ERBB2 (Gly778Asp) decreased its binding to HSP90. Similar results obtained with other kinases such as LIM domain kinase 1 (LIMK1), death-associated protein kinase 1 (DAPK1) and NF-κB-inducing kinase (NIK; also known as MAP3K14) have led to the αC–β4 loop being termed the HSP90-recognition loop[131–133]. However, other regions in or near the kinase domain have also been shown to be important for HSP90 binding[134–136], indicating that the αC–β4 loop is not the sole determinant of association. Direct interaction of the αC–β4 loop with HSP90 has not been shown, and the loop sequence does not distinguish client from non-client kinases on a larger scale[137].

It is therefore plausible that the effects of αC–β4 loop mutations are indirect. An alternative is that HSP90 binding is dictated by the conformation of the kinase. Most kinases reside in well-folded closed conformations until they are activated by specific signals. There are several mechanisms by which kinases are held in the inactive state, but the activated conformation is structurally remarkably conserved[138]. One of the key structural changes during kinase activation is the rotation of the αC helix and its subsequent interaction with the activation loop. Thus, mutations introduced in the αC–β4 loop could affect conformational stability of the αC helix and the activation state of the kinase domain. Indeed, most mutations in the αC–β4 loop alter HSP90 binding and affect kinase activity.

Perhaps the strongest evidence that recognition by HSP90 is related to conformation or stability of the client rather than elements of primary sequence comes from oncogenic kinases. A striking feature of HSP90 is its ability to bind and promote the maturation of numerous mutant protein kinases that would otherwise be degraded. Studies with Rous sarcoma virus showed that although v-Src robustly bound HSP90, its normal cellular counterpart, c-Src (which has ~98% sequence identity[139]), only transiently associated with it[139–141]. c-Src is much more resistant to chemical or thermal denaturation, whereas v-Src is unstable even at normal body temperature *in vitro*[142].

Oncogenic mutations in EGFR and BRAF further support the notion that destabilization of the closed inactive conformation may increase association between HSP90 and the open kinase form. A mutation in EGFR (Leu858Arg) constitutively activates the receptor by destabilizing its inactive conformation[143]. Similarly, the most common activating mutation in BRAF, Val600Glu, renders the inactive conformation energetically unfavourable[144]. Both EGFR Leu858Arg and BRAF Val600Glu strongly depend on HSP90 for stability[145,146], and each associates with HSP90 more than their wild-type counterparts[145,147]. Increased association is not simply due to increased activity. Oncogenic BRAF mutants Gly597Arg and Gly467Val have reduced kinase activity but depend more on HSP90 function than their wild-type counterparts[146]. Interestingly, these oncogenic mutations are also predicted to destabilize the inactive conformation of BRAF[144].

Collectively, these observations suggest several mechanisms by which HSP90 could recognize client kinases. Most simply, HSP90 could directly bind the open active conformation of the kinase. Another possibility is that the open conformation has a higher propensity to unfold into yet another conformation that binds HSP90. Consistent with this hypothesis, low-resolution structural analysis of the cyclin-dependent kinase 4 (CDK4)–HSP90–Cdc37 complex suggests HSP90 binds CDK4 in an extended, perhaps partially unfolded, conformation[13].

As for kinase domains, the elusive structural basis of the steroid hormone receptor binding to HSP90 is now partly understood. HSP90 interacts with the conformationally labile ligand-binding domain (LBD) and not the well-structured DNA-binding domain. In doing so, HSP90 holds the LBD in a conformation that can bind the hormone. Mutational analysis of the glucocorticoid receptor uncovered a heptapeptide in the LBD that is important for both HSP90 association and ligand binding[148–150]. Deletion of the motif abolished both HSP90 and hormone binding, but the region was surprisingly tolerant to point mutations[148,150]. It is therefore unlikely that HSP90 directly binds to the heptapeptide. Instead, deletion of the motif could induce structural rearrangements that impair chaperone–glucocorticoid receptor complex formation.

Molten globule
A partially denatured protein conformation with secondary structure similar to the native fold but without a fixed tertiary structure. Many proteins remain in such a state during folding or under partially denaturing conditions.

Box 3 | **HSP90 and evolution**

In eukaryotes, heat shock protein 90 (HSP90) can markedly influence the phenotypic manifestation of genetic variation. The reservoir of HSP90 function can mask variation[168–171], so that when environmental stress depletes this reservoir, new traits appear in some individuals. In *Drosophila melanogaster*[169], *Arabidopsis thaliana*[170,172] and *Danio rerio*[171], the specific traits seen after HSP90 depletion strongly depended on genetic background. Furthermore, HSP90-dependent changes in life history traits can be mapped to polymorphic loci in *A. thaliana*[172,173]. By expanding the mechanisms by which HSP90 can contribute to producing new heritable phenotypes, HSP90 can affect the heritability of epigenetic traits[174], the induction of new mutations[175] and even cause transposon-induced mutagenesis[123].

HSP90 can also potentiate genetic variation, allowing it to have immediate effects. The best examples are mutated oncogenes, the promiscuous activities of which are enabled directly by HSP90 (REF. 45). However, potentiation can also proceed indirectly. Mutations that provide resistance to azole antifungal drugs can perturb membrane sterol composition[168]. HSP90 drives the protective responses that allow cells harbouring these mutations to grow by chaperoning an unstable phosphatase (calcineurin) that drives a range of protective responses[168].

HSP90-dependent traits can become resistant to environmental change through selection[168,169]. This can occur by the acquisition of new, robust mutations[168] or by the re-assortment and enrichment of initial variation[169]. Adaptive protein variants with altered chaperone dependence can be created during experimental evolution[99,176], but naturally occurring examples of such variation remain elusive. Linkage mapping of HSP90-dependent traits in *A. thaliana* identified many chromosomal regions that harbour such alleles[172] but did not identify specific genes.

Studies in *Saccharomyces cerevisiae* have now filled in many pieces in this puzzle (D.F.J. and S.L., unpublished observations). HSP90 inhibition beneficially affects many phenotypes across diverse genetic backgrounds. The variation involved is genetic and occurs in HSP90 clients, non-clients and even in regulatory sequences. Most strikingly, reducing HSP90 function substantially improved the correlation between genotype and phenotype, suggesting that HSP90 has played an important part in shaping the *S. cerevisiae* genome.

The difficulties in identifying HSP90 binding determinants show how intimately HSP90 is connected to the normal biological regulation of its client proteins. Nearly all mutations that affect HSP90 binding also affect client protein activity, stability or regulation. Interpretation of results both *in vivo* and *in vitro* is therefore challenging. For example, differences in HSP90 binding might arise indirectly from changes in client-binding to a co-chaperone. However, the notion that co-chaperones determine HSP90 specificity merely moves the goalposts and raises the question: what determines the specificity of the co-chaperones? Better understanding of the interplay between different chaperone systems and co-chaperones *in vivo* and *in vitro* is clearly needed.

HSP90 and protein degradation

Although HSP90 generally stabilizes its client proteins, it can also promote protein degradation; for example, of the Von Hippel–Lindau disease tumour suppressor (VHL)[151]. One model for degradation suggests kinetic partitioning is the key. Clients that remain unfolded after many rounds of chaperone binding and release are more likely to encounter the ubiquitin ligases that would mark them for degradation. In this model, HSP90 has a crucial but passive role in protein degradation. However, the discovery of E3 ubiquitin ligases that directly interact with chaperones suggests HSP90 collaborates more actively with the ubiquitin–proteasome system in degrading at least some misfolded proteins[6].

Much research has focused on one such E3 ubiquitin ligase, C terminus of HSP70-interacting protein (CHIP; also known as STUB1), which can ubiquitylate unfolded proteins[152,153]. CHIP interacts with the C termini of both HSP90 and HSP70 through its TPR domain. In cultured cells, CHIP knockdown stabilizes some HSP90 clients, and CHIP overexpression promotes their degradation[154]. Interestingly, HSP90 clients are degraded in CHIP-deficient fibroblasts following HSP90 inhibition (albeit with slower kinetics), indicating that other E3 ubiquitin ligases are involved[154,155]. Because so many E3 ubiquitin ligases are encoded by the human genome (> 600), identifying the ligases involved is a daunting task[156]. However, one promising candidate is the RING and cullin 5 complex (cullins serve as scaffolds for numerous multisubunit E3 ubiquitin ligase assemblies[157]). Cullin 5 knockdown in cultured cells prevents the degradation of the HSP90 clients ERBB2 and hypoxia-inducible factor 1α (HIF1α) following treatment with the HSP90 inhibitor geldanamycin[158], but the specific regulators of ERBB2 and HIF1α degradation is unknown. Many details remain to be elucidated, but it therefore seems that HSP90 clients are not degraded by a single ubiquitin ligase.

Future directions and unsolved mysteries

Three decades of study have uncovered the central importance of HSP90 in countless cell pathways. Nonetheless, despite its central roles in biology and medicine, the inherent complexity of the system has left many aspects of HSP90 function, regulation and structure a mystery.

HSP90 interfaces with diverse protein clients that adopt many different conformations. However, no known sequence motif is shared among them. Precisely how does HSP90 recognize its clients? What is the role of the intrinsic conformational flexibility of HSP90 in this molecular recognition? Many co-chaperones contribute, but how?

Recent structures of full length HSP90 have enabled a much greater understanding of its mechanism of action, and further information should be provided by additional structures of HSP90 in complex with co-chaperones. Given the dynamic nature of HSP90–co-chaperone complexes, it will be especially important to combine the determination of static structures with dynamic techniques. Better understanding of how nucleotide hydrolysis is coupled to HSP90 function is also imperative. However, the greatest challenges are structural determination and dynamic studies of HSP90 in complex with its client proteins.

Answers to these questions will have broad implications not only for evolutionary processes but also for human health. HSP90 couples protein folding to evolutionary change by redrawing the genotype-to-phenotype map (BOX 3). Modulation of HSP90 function also shows promise as a treatment for several cancers, cystic fibrosis and many other human diseases. Comprehensive understanding of how HSP90 function is regulated at all levels may provide even more selective approaches for therapeutic intervention.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
UniProtKB: http://www.uniprot.org
AHA1 | AHR | BRAF | Cdc37 | CDK4 | CHIP | CK2α | Cpr6 | CYP40 | DAPK1 | EGFR | eNOS | ERBB2 | FKBP52 | GroEL | GroES | HIF1α | HOP | HSF1 | Hsp82 | HSP90α | HSP90β | interleukin-6 | interferon-γ | LIMK1 | NF-IL6β | NIK | p23 | p65 | PP5 | Ppt1 | RAB3A | RhoGDIα | Pih1 | σ^{32} | SQUINT | STAT1 | STAT3 | Swe1 | Tah1 | TERT | TOM70 | TTC1 | VHL | WISp39

FURTHER INFORMATION
The Lindquist laboratory homepage:
http://web.wi.mit.edu/lindquist/pub
The Picard Laboratory website: www.picard.ch
BioGRID database: http://thebiogrid.org

ALL LINKS ARE ACTIVE IN THE ONLINE PDF