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Hsp90 – from signal transduction to cell transformation

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Abstract

The molecular chaperone, Hsp90, facilitates the maturation and/or activation of over 100 'client proteins' involved in signal transduction and transcriptional regulation. Largely an enigma among the families of heat shock proteins, Hsp90 is central to processes broadly ranging from cell cycle regulation to cellular transformation. Here we review the contemporary body of knowledge regarding the biochemical mechanisms of Hsp90 and update the most current paradigms defining its involvement in both normal and pathological cell physiology.

Keywords

Hsp90; heat shock protein; molecular chaperone; ATPase; genetic capacitor

Hsp90 defines a family of molecular chaperones that are highly conserved from prokaryotes to eukaryotes [1-5]. Nonessential for normal growth in most bacteria, Hsp90 is abundantly expressed in higher eukaryotes where it has been shown to be necessary for viability [6,7]. It functions as a homodimer that associates with co-chaperones to catalyze the maturation and/or activation of over 100 substrate proteins that are known to be involved in cell regulatory pathways [5]. These 'client proteins' include protein kinases, nuclear hormone receptors, transcription factors, and an array of other essential proteins [8]. While much is known regarding the ATPase-driven conformational cycling of Hsp90, the precise physical effects imparted by this chaperone that serve to activate its substrates are still poorly understood [5].

Hsp90 architecture

Three highly conserved domains comprise the structure of Hsp90. These include the N-terminal domain, responsible for ATP-binding, a proteolytically resistant core domain, and the C-terminal domain that facilitates homodimerization (Fig. 1a) [9]. In eukaryotes, a more variable charged region links the N-terminal domain to the core domain. The length and composition of this linker region is highly divergent among organisms [10]. As no atomic resolution structure for full-length Hsp90 is yet available, the most thorough structural analyses for Hsp90, to date, have been based on crystallographic studies of its individual domains.

A mechanistic understanding of Hsp90 was nebulous until partial sequence homology was recognized between its N-terminal domain and two types of ATP-dependent proteins. These

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included the type II topoisomerases and the MutL DNA mismatch repair enzymes [11]. Structural analyses of the N-terminal domain of Hsp90 revealed that this domain contains an ATP-binding site [12]. Additionally, biochemical studies suggest that transitory interactions between two N-terminal domains of the Hsp90 homodimer occur in an ATP-dependent manner, and this provides the mechanistic basis for an ATPase-driven molecular clamp [13]. Mutations in this region that impair the ability of Hsp90 to either bind or hydrolyze ATP eliminate its chaperone activity [14]. The discovery that the antibiotics, radicicol and geldanamycin, inhibit the Hsp90-dependent activation of numerous regulatory and signal transduction proteins by occupying their ATP-binding sites was a revelation in antitumor research [15]. Indeed, that was the origin of almost a decade of intense efforts focusing on Hsp90 as a therapeutic target for the treatment of cancer.

Biochemical and structural analyses of the core domain of Hsp90 determined that this domain contains a catalytic loop that accepts the γ-phosphate of ATP [16]. This led to the characterization of Hsp90 as a "split ATPase" [16]. Structural and mechanistic similarities shared between Hsp90 and DNA gyrase B serve as the basis for suggesting that the core domain is also involved in the interface of Hsp90 with its client proteins. Strengthening this position, several studies now implicate the core of Hsp90 in its interactions with p53, eNOS, and Akt [17-19].

The advent of the C-terminal crystal structure provided further evidence for the antiparallel dimeric architecture of Hsp90 that had been previously predicted by electron microscopy [20-22]. C-terminal truncations of Hsp90 abolish its ability to hydrolyze ATP, indicating that its dimeric nature is essential for its activity [13]. A highly conserved pentapeptide (MEEVD), present in the C-terminus of eukaryotic Hsp90, is recognized by co-chaperones containing tetratricopeptide repeats [23-25]. Thus, the C-terminal domain is also involved in the formation of active Hsp90 multiprotein complexes.

The structural mechanism for the chaperone activity of Hsp90 has been likened to a 'molecular clamp.' In the absence of bound nucleotide, the N-termini of the Hsp90 homodimer maintain an open-state, facilitating the 'capture' of client proteins (Fig. 1b) [13]. Association with ATP induces modest changes in the conformation of Hsp90 that permit a transitory interaction between the opposing N-terminal domains. This produces the closed-form of Hsp90 where clamping of the substrate protein occurs [13]. It is through this ATPase-driven cycle that Hsp90, with the assistance of several co-chaperones, induces the activation of its 'clientelle' [26].

Hsp90 co-chaperones

Hsp90 is not capable of autonomously functioning as a protein chaperone. Instead, it serves at the core of various multiprotein complexes that incorporate other chaperones, such as Hsp70, and an assortment of co-chaperones [5,27]. The broadest class of Hsp90 co-chaperones are those containing one or more tetratricopeptide repeat (TPR) motifs that interact with the C-terminal domain of Hsp90 [28]. Beyond the conservation of their TPR motifs, these proteins are remarkably diverse, possessing few overlapping biochemical characteristics [28]. Hop/Sti1, for example, facilitates the interaction between Hsp70 and Hsp90 [29], while WISp39 serves as a client protein specificity factor [30]. A number of TPR-containing co-chaperones even convey their own catalytic activities [5]. These include such enzymes as the E3/E4-ubiquitin ligase, CHIP [31], the protein phosphatase, PP5 [32], and several prolyl isomerases [33,34]. It is known that CHIP functions in the targeting of Hsp90 client proteins for proteasome-mediated degradation [31]. However, the biological functions associated with the recruitment of other enzymes to Hsp90 chaperone complexes are still unclear.

Several Hsp90 co-chaperones have been shown to regulate the ATPase-driven molecular clamp cycle associated with its N-terminal domain. While Hop/Sti1, p23, and Cdc37 impair the

progression of this cycle [24,35,36], Aha1 and Cpr6 function to enhance it [35,37]. Because Hop/Sti1 and Cdc37 are both involved with the recruitment of Hsp90 client proteins, their inhibition of the ATPase cycle is thought to permit the loading of client proteins by maintaining the open clamp conformation of Hsp90 [36,38]. Cpr6 is known to subsequently displace Hop/Sti1 by competing for the C-terminal TPR-recognition motif of Hsp90, thereby permitting progression of the clamp cycle [24]. The Hsp90 activation potential of Aha1 is achieved through extensive associations along the core domain of Hsp90 that induce conformational changes within its catalytic loop. These adjustments place the active site of the Hsp90 loop in better proximity for the acceptance of the γ-phosphate of ATP [39].

Hsp90 client proteins

The most detailed understanding of the effects of Hsp90 on its client proteins has been gleaned from its involvement with the maturation of steroid hormone receptors. Steroid receptors must be maintained in a labile conformation that allows them to be rapidly activated in the presence of their cognate ligand [40]. Hop1/Sti1, by virtue of its ability to bind Hsp70 and Hsp90 in tandem, facilitates the transfer of Hsp70-bound receptors to the open form of Hsp90. The Hsp90 system then induces subtle alterations in the conformation of the bound steroid receptor that enhances its affinity toward its respective ligand [41].

Protein kinases comprise the most prevalent group of Hsp90 client proteins. The co-chaperone Cdc37 is known to interact both with protein kinases and Hsp90, thereby delivering client kinases to the Hsp90 chaperone complex [42,43]. Bound to Hsp90, the client kinases are stabilized and remain in a receptive but inactive state while awaiting appropriate signals [42]. The details of the Hsp90-protein kinase chaperone system are still under investigation.

Beyond its specific *in vivo* role in chaperoning authentic client proteins, Hsp90 has long been noted for its capacity to impede the *in vitro* aggregation of a broad range of non-specific proteins induced to express in *E. coli* [44]. Table 1 lists proteins for which we have used strains of *E. coli* that over-express Hsp90 (Plus90α™; Plus90β™; Expression Technologies Inc., San Diego, CA) to prevent aggregation during expression [45-52]. This illustrates the structural and functional disparity among the *in vitro* clientele of Hsp90. Figure 2 demonstrates the dramatic increase in soluble protein product rendered in Hsp90-over-expressing strains of *E. coli*. While the selectivity of Hsp90 for its *in vivo* client proteins points to a highly specific mechanism, the architectural variation among its substrates, both *in vivo* and *in vitro*, implicate a far more general mechanism. In this way, Hsp90 remains an enigma among heat shock proteins.

Chaperoning tumorigenesis

The essential roles that Hsp90 fulfills in the normal physiology of healthy cells are even more critical for the viability of transformed cells. Hsp90 is absolutely essential for the stabilization/maturation of nuclear hormone receptors, transcription factors, and protein kinases that are commonly misregulated during tumorigenesis [8]. It also serves to buffer the effects of transformation by preventing the aggregation of aberrantly expressed proteins, whose accumulation would otherwise result in toxic stress signals and progression to programmed cell death [53]. As many of the client proteins of Hsp90 are linked to growth signal pathways, Hsp90 is viewed as key player in the subversion of normal cells toward unrestrained proliferation. Amplifying the corruptive potential of Hsp90 is its ability to facilitate the evolution of neoplastic clones by stabilizing many of the mutated proteins that are often associated with cancerous lesions, including p53, Bcr-Abl, and v-Src [4,53]. For this reason, Hsp90 is thought to be especially crucial in the development of tumors that result from the inactivation of DNA repair pathways, in which there are extensive pools of diversely mutated proteins (Fig. 3).

The earliest studies highlighting the antitumor capacities of geldanamycin and radicicol credited their abilities to impair the activity of oncogenic protein kinases such as ErbB-2 and v-Src [54]. It was later shown that the biological target of these drugs is actually Hsp90, and that their use blocks the Hsp90-dependent activity of Raf-1, Cdk4, Src-family kinases, and many other oncogenic targets [53,54]. Since then, immense progress has been made in the development of pharmacological agents that act as inhibitors of Hsp90. In addition to their role in cancer therapy, these drugs will undoubtedly reveal new insights into the involvement of Hsp90 in diverse physiological processes.

Hsp90 as a 'genetic capacitor'

By chaperoning mutated clients, Hsp90 facilitates the accumulation of mutant proteins [53, 55]. Beyond the implications this may have in tumorigenesis, the stabilization of proteins that may otherwise be degraded allows Hsp90 to act as a buffer for phenotypic change [53,55]. Studies in *Drosophila* and *Arabidopsis* have revealed that Hsp90 curbs phenotypic variations under ordinary conditions, allowing their manifestation only when Hsp90 is functionally inert [56,57]. As a chaperone for many proteins that lie along broad-reaching signal cascades, the function of Hsp90 is central to key developmental processes [4,5]. Therefore, when the activity of Hsp90 is compromised due to environmental stress or the application of Hsp90 inhibitors, the effects are often pleiotropic [58]. In this capacity, Hsp90 has been portrayed as a 'capacitor for evolution' [57]. Since a significant portion of mutated proteins stabilized by Hsp90 likely result from genetic mutations, Hsp90 has also been described as a 'genetic capacitor' [58]. This adds to the ever-increasing convolution involved with the translation of genotype to phenotype.

Conclusions

From its crucial roles in signal transduction to transformation to genetic capacitance, Hsp90 is a ubiquitous molecular chaperone that influences an expansive array of cellular events through its broad range of protein clientele. Hsp90 has been the focus of intense research for the past 20 years, resulting in the establishment of several overlapping paradigms stemming from the ATP-dependent chaperoning cycle of Hsp90. In spite of this immense progress, many challenges remain. For example, while much is known regarding the ATPase-driven conformational cycling of Hsp90, the precise physical effects imparted by this chaperone that serve to activate its substrates are still poorly understood. In addition, the currently known repertoire of Hsp90-dependent proteins is far from complete. A more comprehensive listing and characterization of its clients will undoubtedly reveal the vast-reaching governance wielded by Hsp90 as an intermediate custodian of far-reaching physiological processes. As the target for several promising lines of cancer therapeutics, Hsp90 is certain to remain the focus of intense research for many years to come.

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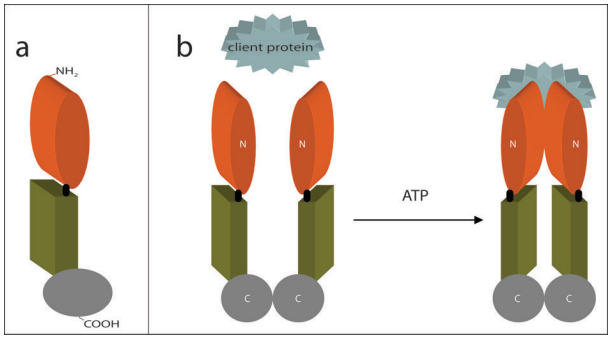

Figure 1. The structure of Hsp90 and its ATP-dependent molecular clamp

(a) Schematic representation of Hsp90. The Hsp90 monomer is comprised of three domains: the N-terminal domain responsible for ATP-binding (orange), a core domain (green), and a C-terminal domain that facilitates homodimerization (gray). In eukaryotes, a short charged region links the N-terminal and core domains (black). (b) ATP-driven molecular clamp cycle of Hsp90. In the absence of bound nucleotide, the C-termini (C) of two Hsp90 monomers interact to maintain an antiparallel dimer (left). Concurrently, the N-termini (N) of the Hsp90 homodimer preserve an open-state, facilitating the capture of client proteins (left). On the right, association with ATP induces modest changes in the conformation of Hsp90 that permit a transitory interaction between the opposing N-terminal domains. This produces the closed-form of Hsp90 where clamping of the substrate protein occurs.

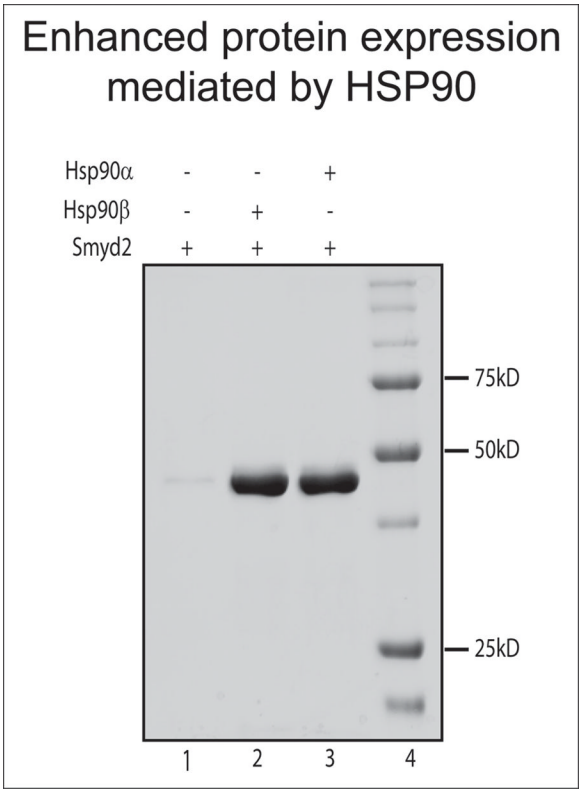

Figure 2. Hsp90α and Hsp90β enhance the solubility of proteins expressed in *E. coli*

There are two isoforms of Hsp90 (Hsp90α and Hsp90β) which share 85% identity and maintain virtually indistinguishable functional properties [59]. Here, Hsp90 isoform-expressing *Escherichia coli* strains produce higher yields of soluble Smyd2. Wild type (lane1) and Hsp90 isoform-expressing (lanes 2 and 3) *E. coli* strains were transformed with a Smyd2 expression construct [45]. Equal amounts (2.5 g wet cell paste) of cultured *E. coli* cells were used for isolation and purification of Smyd2. The migration of molecular weight markers (in kiloDaltons) is indicated. The depicted results are representative of the enhanced solubility achieved with numerous proteins (Table 1) in $Hsp90^{+}$ strains.

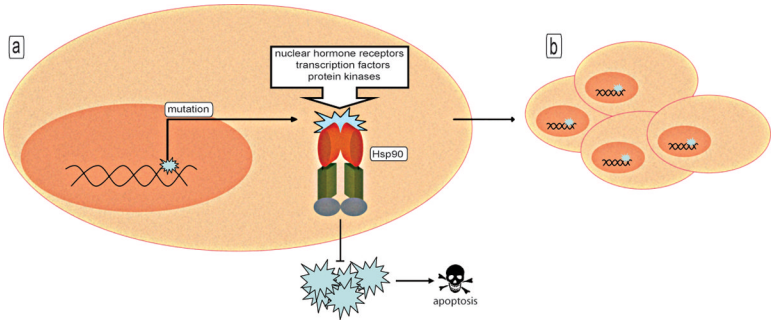

Figure 3. Role of Hsp90 in chaperoning tumorigenesis

(a) Hsp90 stabilizes many mutated proteins that mediate cell transformation and prevents the aggregation of aberrantly expressed proteins (blue), which would otherwise result in toxic stress signals leading to the progression of apoptosis. (b) The chaperoning capacity of Hsp90 facilitates the evolution of neoplastic clones.

Table 1

Partial list of recently established Hsp90 *in vitro* client proteins

Protein	Function
Smyd2	catalyzes methylation of p53 (K370) and histone H3 (K36) [45,48]
Smyd3	catalyzes methylation of histone H3 (K4) [47]
RNA polymerase II	RNA polymerase [46]
FKBP38	calcineurin inhibitor [49]
MICAL	cytoskeletal regulation [50]
skNAC	muscle-specific transcription factor [51]
Bcl2	regulator of apoptosis [52]

Hsp90 stabilizes over 100 client proteins [5]. Proteins shown in this table are recently established *in vitro* clients that we have observed in our own laboratory, based on their enhanced solubility and yields in the Hsp90 over-expressing strains of E. coli. Figure 2 illustrates representative results.