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Clients Place Unique Functional Constraints on Hsp90

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Summary

Hsp90 is required for activation and stabilization of numerous client proteins, yet the functional requirements of individual clients remain poorly understood. Utilizing yeast growth assays and mutational analysis, Mishra and colleagues explore the constraints placed on Hsp90 by distinct clients and the relationship between these constraints and overall yeast fitness.

Keywords

Hsp90; molecular chaperone; chaperone-mediated client maturation

Heat Shock Protein 90 (Hsp90) is a dimeric chaperone protein that is essential in eukaryotes. Approximately 10% of cellular proteins require Hsp90 for their activation and stability; these proteins are termed Hsp90 clients. Many clients have functions related to cell signaling and growth, making cancer cells highly dependent on functional Hsp90. Hsp90 also plays a role in diseases other than cancer including Alzheimer's disease, other neurodegenerative diseases, inflammation and infectious diseases [1]. The stability or activity of some client proteins has been associated with distinct disease states, yet the degree of dependence of individual clients on Hsp90 ATPase activity is not well understood [2]. While pharmacologic inhibition of Hsp90 results in client destabilization and degradation, making the chaperone an intriguing drug target, no Hsp90 inhibitor has yet been approved for clinical use. Although some client-specific requirements with respect to Hsp90 have been identified, one of the limitations of current Hsp90 inhibitors, most of which are directed to the N-domain ATP binding pocket, is a lack of client (or even client subset) specificity [3].

Hsp90 is comprised of three domains: an N-terminal ATP-binding domain, a middle client-binding domain and a C-terminal dimerization domain. The N-terminal domain contains a solvent exposed, highly twisted eight-stranded β -sheet, a covered α -helical region that forms a deep pocket, and a loop that can cover the pocket, known as the lid (Figure 1). A central role of the N terminus is to bind ATP within the unique Gyr-B like pocket, thereby initiating a complex series of conformational changes that result in the acquisition of ATPase activity

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[4]. Thus, upon binding ATP, the lid closes over the nucleotide to trap it in the pocket. This closure is followed by Hsp90 N-terminal dimerization and interaction of the N terminus with the middle domain, leading to the hydrolysis and release of the bound nucleotide. Despite having inherently low ATPase activity, ATP binding and hydrolysis is essential for Hsp90 function [5]. The N-terminal domain is also the most evolutionarily conserved region of Hsp90, suggesting its specific sequence is required for Hsp90 function. In support of this hypothesis, previously characterized mutations of conserved residues within the N-terminal ATP binding pocket have led to complete disruption of Hsp90 activity and cell lethality (e.g., D79N) [5].

The N-terminal domain of Hsp90 binds distinct client and co-chaperone proteins [2]. Despite the obvious necessity of the N-terminal region for Hsp90 function, specific dependence of individual clients on the ATP hydrolysis rate or amino acid sequence of this region is not clear. To better understand this relationship, Mishra and colleagues utilized a mutational scanning approach – sequentially changing each amino acid within the Hsp90 N-terminal domain in otherwise wild type yeast -and examined the impact on fitness [6]. Not surprisingly, mutations displaying the greatest fitness defects were clustered around the region of ATP interaction, specifically those amino acids that are in proximity to, or interact directly with, the phosphates of ATP. Interestingly, however, mutations that had variable effects on ATPase activity supported yeast viability, suggesting a complex relationship between hydrolysis rate and Hsp90 function. Indeed, the Hsp90-D79E and -D79C mutations, in contrast to D79N (see above), were both able to bind and hydrolyze ATP while minimally impacting fitness. Utilizing these mutants, Mishra and colleagues then examined chaperoning activity toward two well-characterized but structurally distinct Hsp90 clients, the glucocorticoid receptor (GR) and v-SRC kinase. The more ATPase compromised, but still viable, D79C was able to productively chaperone GR, but not v-SRC, suggesting that these distinct clients place unique demands on Hsp90 ATPase activity.

Based on these perhaps unexpected results, Mishra and colleagues further explored whether the distinct structural constraints placed on Hsp90 N-domain function by GR and v-SRC relate to requirements for Hsp90 to support yeast viability. To accomplish this, they assessed client activity under two conditions: (1) when native amino acids in Hsp90 were replaced with amino acids that caused large physical changes but small fitness defects, and (2) when native amino acids were replaced with amino acids that caused small physical changes but large fitness defects. Almost half of the N-domain mutations isolated in this panel exhibited strong client-specific effects (see Figure 1). Mutations with little impact on fitness supported efficient GR activity, but many of these same mutations were deficient in chaperoning v-SRC. Hsp90 mutants that caused large fitness defects universally were unable to properly chaperone v-SRC, but many retained the ability to chaperone GR. These data suggest that GR places relatively few constraints on Hsp90 ATPase domain activity or sequence, while v-SRC, in contrast, appears to be much more demanding when compared to the Hsp90 clients in yeast (still unidentified) that are necessary for yeast viability. In fact, mutations that severely impacted v-SRC had variable effects on the yeast client kinase Ste11, demonstrating that even clients of the same class (e.g., kinases) place distinct constraints on Hsp90 ATPase domain function.

The involvement of many Hsp90 clients in disease continues to provide a rationale for pursuing Hsp90 as a drug target. However, this endeavor is hampered by the growing realization that current Hsp90 inhibitors are generally not able to take advantage of the highly variable chaperone dependence of the large and diverse Hsp90 clientele, making it difficult to inhibit the maturation of specific clients while minimally impacting others. By helping to further clarify the distinct structural and functional constraints that individual clients place on Hsp90, the approach provided by Mishra and colleagues will facilitate the possibility of generating client-specific inhibitors of this medically important molecular chaperone.

References

1. Taldone T, et al. Selective targeting of the stress chaperome as a therapeutic strategy. *Trends Pharmacol Sci.* 2014; 35(11):592–603. [PubMed: 25262919]
2. Zuehlke A, Johnson JL. Hsp90 and co-chaperones twist the functions of diverse client proteins. *Biopolymers.* 2010; 93(3):211–7. [PubMed: 19697319]
3. Karagoz GE, Rudiger SG. Hsp90 interaction with clients. *Trends Biochem Sci.* 2015; 40(2):117–25. [PubMed: 25579468]
4. Prodromou C, et al. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell.* 1997; 90(1):65–75. [PubMed: 9230303]
5. Panaretou B, et al. ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. *EMBO J.* 1998; 17(16):4829–36. [PubMed: 9707442]
6. Mishra, P., et al. Systematic Mutant Analyses Elucidate General and Client-Specific Aspects of Hsp90 Function. *Cell Rep.* 2016. DOI: <http://dx.doi.org/10.1016/j.celrep.2016.03.046>

Figure 1. Hsp90 N-terminal Domain Structure
(A) A model of the N-terminal domain of Hsp90, including its α -helices (orange), β -sheets (magenta), lid (green), client-specific mutations (red) and client-unspecific mutations (cyan) while bound to the Hsp90 inhibitor Geldanamycin (yellow), was created using the MacPyMOL program and the 1YET.pdp file. (B) A table representing the client-specific and client-unspecific mutations, adapted from Mishra et al. [6].