CellPress

Picky Hsp90—Every Game with Another Mate

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http://dx.doi.org/10.1016/j.molcel.2017.09.013

In this issue of *Molecular Cell*, Sahasrabudhe et al. (2017) present a dramatically renovated functional cycle for the molecular chaperone Hsp90, which stimulates re-thinking of the mechanism of this vital protein folding machine.

The molecular chaperone Hsp90 plays an irrefutable role in the folding, maturation, and degradation of a large number of client proteins. In the eukaryotic cytosol, Hsp90 possesses a wealth of options to adjust its activity by specific co-chaperones. More than 20 co-chaperones have been identified so far that assist Hsp90 in this heroic fight for balanced cellular homeostasis, but it is unclear if all of them are needed for maintenance of every client (a list of verified co-chaperones and clients is maintained at https://www.picard.ch/downloads/Hsp90interactors.pdf; Li et al. 2012; Picard, 2012). In this issue of *Molecular Cell*, Sahasrabudhe et al. (2017) perform a comprehensive analysis of the co-chaperone requirements for two key client families, with astonishing results.

The Hsp90 chaperone machinery fosters the folding, maintenance, and degradation of several hundred client proteins, which belong to various sequentially and structurally unrelated protein classes and include many regulatory proteins. The need for Hsp90 is inversely correlated with stability of the client (Taipale et al., 2012). A large number of kinases, transcription factors including many steroid hormone receptors, and even disordered proteins are Hsp90 client proteins. Many of them are involved in serious maladies, such as cancer, Alzheimer's disease, and cystic fibrosis.

Hsp90 is a homodimer that typically assists its substrate proteins late on the folding path (Karagöz and Rüdiger, 2015). ATP binding facilitates the transient dimerization of the N-terminal domains, which induces a switch from an extended, open, C-terminally dimerized conformation to an N- and C-terminally linked closed conformation (Pearl and Prodromou, 2006). A working ATPase cycle is required for client transfer from the Hsp70 to the Hsp90 system and therefore essential to Hsp90s chaperone function (Kirschke et al., 2014; Smith et al., 1992). Co-chaperones modulate the ATPase and functional cycle of Hsp90 by adjusting its N-terminal dimerization and ATP-hydrolysis rate, controlling client targeting or acting as adaptor proteins. Despite the intensive research directed on deciphering the working mechanism of Hsp90, we still have limited understanding of the molecular principles of its client modeling capacity. How do clients benefit from ATP hydrolysis? How does the opening and closing of Hsp90 contribute to folding and maturation of its substrates? Until now, co-chaperones seemed to offer attractive solutions for this inextricable puzzle.

In their study, Sahasrabudhe et al. (2017) set out to understand the necessity of co-chaperones for client activity using yeast as model organism. They systematically deleted (or knocked down) 12 Hsp90 co-chaperones and followed the change in the activity of five steroid hormone receptors (SHRs) and Src kinases. This approach allowed them to compare the co-chaperone requirements within but also between two different client families. Their work resulted in a radically simplified Hsp90 cycle with dramatically reduced influence for co-chaperones.

Remarkably, only two of the co-chaperones, p23 and Sgt1, support maturation of all clients tested. It is interesting to note that both proteins have the same fold, but they bind to different positions on the Hsp90 surface, and they do not complement each other (Zhang et al., 2008). Whether this is just a coincidence or has a mechanistic background remains unclear. As expected, the kinase v-Src also benefits from the kinase-specific substrate-targeting factor Cdc37 but, intriguingly, as well as from the Hsp70 adaptor protein Hop. This suggests that the Hsp70 chaperone may also have a role in kinase targeting of Hsp90.

While v-Src seems to have only activating co-chaperone partners, in the case of SHRs, Sahasrabudhe et al. (2017) found more co-chaperones that suppress than activate maturation, including the ATPase stimulator Aha1. The large number of retarding co-chaperones suggests that Hsp90 may be more active in promoting client maturation than what is required for effectively running the cell. Overactivity may be a challenge for the eukaryotic cytosol, where many clients of Hsp90 cannot complete folding unless they bind a small molecule (the hormone in case of SHRs) or become activated (kinases). Retarding co-chaperones may be key to dealing with this issue, as they decelerate the functional cycle, which may allow Hsp90 to hold SHRs in a non-native state until the hormone binds.

Intriguingly, Hsp90 partners with a different set of co-chaperones for each SHR, indicating it is not the fold of the client itself that determines the co-chaperone need. When Sahasrabudhe et al. (2017) compared five structurally homologous SHR, they revealed astonishing differences. Every co-chaperone is consistently either activating or deactivating, but each SHR has its own co-chaperone signature. For example, only glucocorticoid receptor benefits from Hop, Aha1 represses mineralocorticoid receptor but

not progesterone receptor, and only androgen receptor is deactivated by Cpr7 and Pih1. Also, limited proteolysis revealed that choice of the co-chaperones can influence structural integrity and protein stability of the client. How are needs for and selection of the co-chaperone determined? And what does a co-chaperone do to a particular substrate protein? Answers to these questions remain open until we have molecular insights in the mechanism of how co-chaperones tune the Hsp90 engine.

Notably, co-chaperones do not run the engine. The impact of their deletion is surprisingly mild, as client activity is typically affected just by only a factor of two to three. For comparison, in the Hsp70 cycle, co-chaperones (J-proteins and nucleotide exchange factors) stimulate its activity by three orders of magnitude! Thus, the Hsp90 co-chaperones subtly fine-tune Hsp90 activity. There are many of them, but they are not integral parts of the chaperone engine.

Recent years have seen increasing efforts to allocate more and more co-chaperones to specific steps in the functional cycle of Hsp90. Many open questions remain regarding the roles of Hsp90 co-chaperones: Why do you need a plethora of structurally unrelated co-chaperones to modulate Hsp90? After all, Hsp90 switches between just a limited number of states. Often binding of one co-chaperone excludes simultaneous binding of others—do these co-chaperones fight for attention of Hsp90? And how does Hsp90 pick the winner? At the least, we learn from Sahasrabudhe et al. (2017) that the role of Hsp90 strengthened: it shows a cat-like character, a solitary predator that firmly holds on to the yarn, even if occasionally picking a fellow mate to join the game (Figure 1). Shall we be surprised? Looking beyond the membranes that enclose the vertebrate cytosol, the answer is no. Both the endoplasmic reticulum and mitochondria have Hsp90 paralogs; however, paralogs of none of the co-chaperones have yet been found in these compartments. Nor does *E. coli* Hsp90 ever require the help of any homologous co-chaperones. Thus, from an evolutionary point of view, it makes sense that the basic running of the engine does not require co-chaperones. It reminds us that the most important obstacle to deciphering co-chaperone action is still the limited understanding of the Hsp90 mechanism itself.

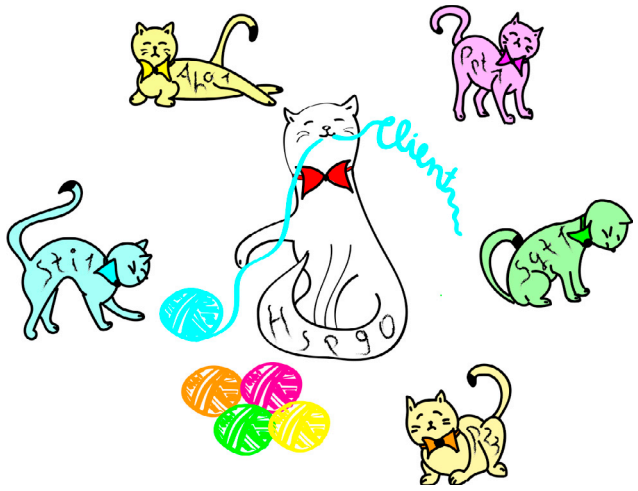

Figure 1. Master of the Yarn
Hsp90 takes center stage to play with its client, holding firmly onto the yarn itself and only occasionally inviting a select co-chaperone to join.

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