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https://doi.org/10.1080/15548627.2024.2323259

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The HSP90 chaperone code regulates the crosstalk between proteostasis and autophagy

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ABSTRACT

Proteostasis, the maintenance of proper protein folding, stability, and degradation within cells, is fundamental for cellular function. Two key players in this intricate cellular process are macroautophagy/autophagy and chaperoning of nascent proteins. Here, we explore the crosstalk between autophagy and the HSP90 chaperone in maintaining proteostasis, highlighting their interplay and significance in cellular homeostasis.

Abbreviation: HSP90: heat shock protein 90; PTMs: post-translational modifications

ARTICLE HISTORY
Received 26 January 2024
Revised 13 February 2024
Accepted 21 February 2024

KEYWORDS
Atg1; chaperone; chaperone code; co-chaperone; phosphorylation; ULK1

Macroautophagy/autophagy primarily functions in response to cellular stressors such as nutrient deprivation or protein aggregation. In response to starvation, autophagy recycles cellular material by non-selectively engulfing intracellular components. In other cases, autophagy can selectively target specific components for degradation, such as damaged organelles or aggregated proteins, by a process known as selective autophagy. For instance, mitophagy selectively removes damaged mitochondria, promoting cellular health and energy production. One of the pivotal roles of autophagy in proteostasis is the clearance of misfolded or aggregated proteins. By selectively engulfing these proteins into phagophores, autophagy prevents the accumulation of toxic aggregates that can disrupt cellular function and lead to diseases like neurodegeneration or cancer.

HSP90 (heat shock protein 90) is a central player in the cellular chaperone network, ensuring folding, stability and activity of client proteins. HSP90 functions in concert with co-chaperones and post-translational modifications (PTMs) to maintain integrity and functionality of the proteome. This is collectively referred to as the chaperone code. One intriguing aspect of the role of HSP90 in proteostasis is its involvement in regulating protein stability. HSP90 helps stabilize many signaling proteins, such as kinases and transcription factors, which are crucial for various cellular processes. HSP90 acts as a "buffer" that prevents these proteins from undergoing degradation so they are available when needed. Autophagy and HSP90 do not operate in isolation; instead, they exhibit a fascinating interplay. HSP90, under stressful conditions, can stabilize client proteins involved in autophagy regulation. For instance, HSP90 modulates the activity of MTOR (mechanistic target of rapamycin kinase), a critical regulator of autophagy. By doing so, HSP90 indirectly influences the autophagic response. Conversely, autophagy can target HSP90 clients for degradation. This mechanism is essential for eliminating damaged chaperone machinery components or clients that are beyond repair. Such crosstalk ensures that only functional chaperones are available to assist in proteostasis, thereby preventing pathological manifestations such as cancer.

Unraveling the complex regulatory mechanisms of HSP90 and its impact on cellular processes requires understanding the chaperone code and the functional consequences of the PTMs. One significant PTM identified in recent work is the phosphorylation of a conserved serine site, yeast Hsp82 S25/human HSP90-alpha S39, within the amino-terminal domain of Hsp82/HSP90. The phosphorylation of this site is catalyzed by the autophagy activating kinase Atg1/ULK1. Notably, this serine/threonine kinase itself is an HSP90 client protein, and inhibition of HSP90 leads to the degradation of Atg1/ULK1. This finding highlights the interplay between HSP90 and maintenance of cellular regulatory pathways.

Our recent findings indicate that HSP90 chaperones Atg1/ULK1 under steady-state conditions, holding the kinase in an inactive state; however, upon stimulation by environmental cues, Atg1/ULK1 phosphorylates HSP90 [1]. This PTM subsequently inhibits HSP90 chaperone activity and leads to the dissociation of the Atg1/ULK1-Hsp82/HSP90 complex, allowing Atg1/ULK1 to become activated and initiate autophagy (Figure 1). This intriguing interplay between Hsp82/HSP90 and Atg1/ULK1 underscores the dynamic nature of cellular proteostasis and its responsiveness to external stimuli.

To further investigate the role of this phosphorylation event at the protein structural level, the impact of ATP on the ULK1-HSP90 complex was assessed using limited proteolysis-mass spectrometry/LiP-MS. This analysis identified a conformotypic peptide adjacent to the activation and catalytic loops of Atg1/ULK1. This conformational change likely influences the kinase activity of Atg1/ULK1 following phosphorylation and dissociation from HSP90. Mutation of the conformotypic peptide abrogates Atg1/ULK1 autophosphorylation, therefore revealing the molecular basis of this regulatory process.

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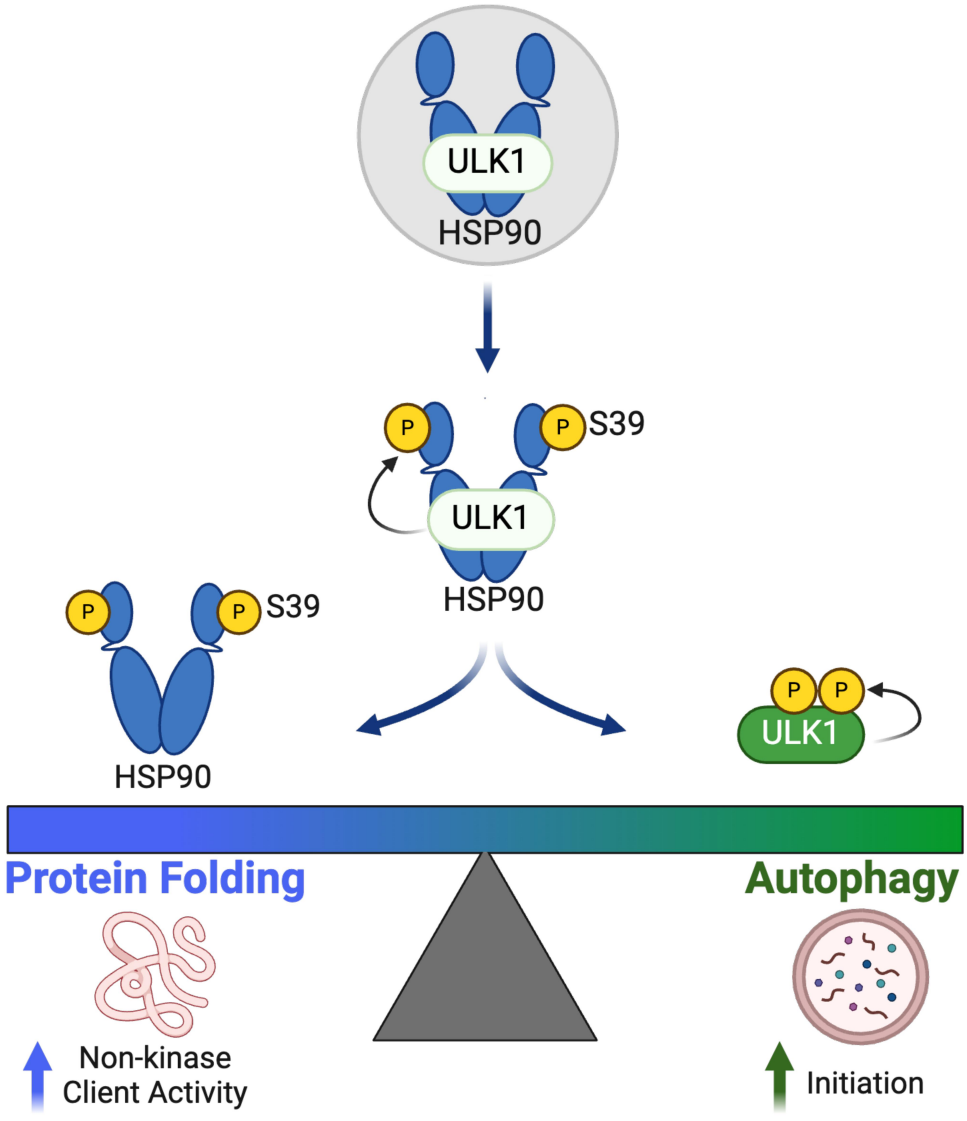

Figure 1. ULK1/Atg1-mediated phosphorylation of HSP90/Hsp82 regulates its chaperone function and consequently controls the stability and activity of its client proteins including ULK1/Atg1. This process leads to autophosphorylation of ULK1/Atg1 and subsequent activation of autophagy.

In order to understand the impact of this phosphorylation on Hsp82/HSP90 chaperone function, we mutated S25 to nonphosphorylatable alanine or phosphomimetic glutamic acid and evaluated Hsp82 client activity. Interestingly, these mutations have opposite effects on the activity of kinase and non-kinase clients. Nonphosphorylatable Hsp82^{S25A} leads to hyperactivity of typical kinase clients, but negatively affects non-kinase client activity, whereas Hsp82^{S25E} has the opposite effect (Figure 1). These results suggest that the phosphorylation status of Hsp82 at S25 plays a role in determining the chaperone's client specificity.

The complexity of Hsp82/HSP90 PTMs is highlighted in this study, emphasizing the importance of conducting detailed investigations into the consequences each PTM on chaperone function and its regulation of various cellular processes [1]. Hsp82/HSP90 involvement in multiple cellular pathways, particularly signaling pathways, underscores the significance of understanding how PTMs influence its function in a context-specific manner. Furthermore, Hsp82/HSP90 is subject to additional regulation by co-chaperones, many of which are themselves regulated by PTMs. This adds another layer of complexity to the overall regulation of chaperone function within the cell.

In the context of cancer, where dysregulation of HSP90-dependent pathways is common, HSP90 inhibitors have been extensively studied for potential therapeutic applications. The successful development of HSP90 inhibitors, such as pimitespib (Jeselhy®, TAS-116), underscores the clinical relevance of understanding the direct role of HSP90 in cellular processes and how its function is regulated. A comprehensive understanding of HSP90 and its regulatory mechanisms may provide crucial insights into the optimal use of HSP90 inhibitors for the treatment of diseases like cancer. Overall, this study sheds light on the intricate chaperone function of HSP90 and its role in cellular proteostasis, thereby opening doors for further therapeutic applications [1].

In conclusion, autophagy and the HSP90 chaperone are indispensable players in the maintenance of proteostasis, ensuring proper protein folding, stability, and degradation within cells. Their dynamic interplay orchestrates a delicate balance that safeguards cellular health. Dysregulation of either process can lead to the accumulation of toxic protein aggregates and the onset of various diseases, including neurodegenerative disorders and cancer. Understanding the intricate relationship between autophagy and HSP90 is not only crucial for advancing our knowledge of cellular biology but also holds promise for the development of novel therapeutic strategies targeting proteostasis-related diseases.

Acknowledgements

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This work was also supported with funds from the SUNY Upstate Medical University, Upstate Foundation. Schematics in Figure 1 was created with BioRender.com.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Institute of General MedicalSciences of the National Institutes of Health (NIH) under [Award Number R35GM139584] (M.M.).

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