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SPOTLIGHT

Sis1 delivers the State of the Union

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The heat shock response (HSR) is a gene expression program that protects cells from heat and proteotoxic stressors. In this issue, Feder et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202005165) show that subcellular relocalization of the cochaperone Sis1 drives the HSR by de-suppressing the transcription factor Hsf1.

In 1962, Ferruccio Ritossa observed that fly chromatin experienced stereotyped rearrangements in response to heat (1). This early evidence of stress-induced gene regulation was part of the eukaryotic heat shock response (HSR), an essential and conserved stress response for maintaining protein quality in response to environmental and physiological stresses (2). The HSR is primarily mediated by the protein heat shock factor 1 (HSF1), which drives transcription of molecular chaperones such as Hsp90, Hsp70, Hsp60, and other protein quality control (PQC) factors that detect, refold, or help degrade misfolded proteins (2). Insufficient PQC has been implicated in multiple diseases, including Parkinson's disease, Alzheimer's disease, frontotemporal dementia, and Huntington's disease, as well as in aging (3). Conversely, in tumors, proteome imbalances caused by aneuploidy as well as rapid cellular growth creates a cancer-specific dependence on PQC (4). As a result, aberrantly high Hsf1 activity is a major survival strategy for cancer cells. Modalities for either activating or suppressing the HSR thus have therapeutic implications. Yet despite its early discovery and the creative contributions of many scientists, our understanding of the fundamental regulatory mechanisms that control the HSR remains incomplete.

The "chaperone titration" model of Hsf1 activation posits that in the absence of stress, chaperones interact with Hsf1 to repress its activity (5, 6). Under conditions of elevated stress, chaperone clients sequester them away from Hsf1, thereby derepressing202012783 Hsf1 and inducing the HSR (5, 6). Yet applying this model to observations about the HSR reveals several issues that deserve closer scrutiny. First, the clients of major chaperone systems proposed to regulate Hsf1 (e.g., Hsp70 and Hsp90) exist throughout the cell, whereas Hsf1 must be nuclear in order to drive transcription. How do changes in cellular protein quality determine the transcriptional response of Hsf1? Second, the time between heat shock and onset of HSR is <2 min. How are changes in the state of the cytosol relayed to Hsf1 in a quick and timely manner? Finally, the levels of major chaperones proposed to regulate Hsf1 are ∼1,000-fold higher than Hsf1, and thus a law of mass action–based model does not predict the observed dynamics of Hsf1 activation under stress (7). How then does Hsf1 activation via chaperone displacement overcome stoichiometric imbalances?

To address these mysteries, Feder et al. (8) first surveyed chaperones that have been previously implicated in repressing HSR. They engineered conditional depletions in budding yeast of candidate chaperones—Hsp70, Hsp90, and Hsp40/J proteins—from the nucleus and compared Hsf1 activation under normal growth conditions and heat shock. Consistent with their previous study (5), they identified Hsp70 as a potent suppressor of Hsf1 and found that an Hsp40 protein, Sis1, also repressed Hsf1. Sis1 has previously been shown to act as a shuttling factor for quality-control substrates (9, 10). Yet transcriptional analysis suggested that nuclear depletion of Sis1 specifically activated Hsf1 without activating other stress-response pathways. These observations suggested a direct role for Sis1 in Hsf1 regulation.

Immunoprecipitation (IP) of Hsf1 did not reveal a stable interaction with Sis1. Instead, the authors hypothesize that Sis1 transiently interacts with Hsf1 to promote formation of the Hsf1–Hsp70 complex. Indeed, deletion of J-domain from Sis1 or alterations in the highly conserved His33-Pro34-Asp35 motif of J-domain rendered it incapable of repressing Hsf1 and HSR, suggesting that the cochaperone activity of Sis1 is critical for Hsf1 suppression. Purification of Hsf1 from cells with depleted levels of nuclear Sis1 yielded lower amounts of Hsp70 proteins than IPs from Sis1-intact cells, providing further evidence that Sis1 promotes Hsp70–Hsf1 interaction under nonstress conditions. To identify the heat shock–induced interactome of Sis1, the authors immunoprecipitated Sis1 from heat shocked cells and found that Sis1 associates with a host of nucleolar, cytosolic, and ER-resident proteins as well as quality-control factors, suggesting a major stress-induced subcellular reorganization of Sis1. Consistent with this, live-cell fluorescence imaging revealed that Sis1 relocalizes from the nucleoplasm to the nucleolar periphery within 2 min of heat shock, followed by formation

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of a network of Sis1-containing cytosolic and ER-associated clusters.

To probe which proteins misfold during heat shock and recruit Sis1, the authors blocked translation using the ribosome elongation–inhibiting drug cycloheximide. Pretreatment with cycloheximide before heat stress reduced the formation of Sis1 inclusions throughout the cell, suggesting that newly synthesized proteins misfold under heat shock and are targeted by Sis1. Other clues for Sis1 clients came from heat shock–induced Sis1 association with the proteasome and Cdc48, an AAA ATPase that targets substrates for proteasomal degradation in multiple PQC pathways, including ER-associated degradation and ribosome-associated quality control. It is thus an attractive hypothesis that relocalization of Sis1 upon heat stress is part of a pan-cellular strategy to detect defects in cytosolic, nucleolar, ER, and ribosome protein quality and respond by triggering the HSR.

How cells survey cytoplasmic events to modulate Hsf1 activity in the nucleus has been a long-standing mystery. Feder et al.'s work expands upon existing models of Hsf1 activation and proposes a general mechanism for stress-induced gene activation that may be relevant to other pathways regulated by chaperones. The study establishes the centrality of Sis1 in activating the HSR by serving as a key interrogator of multiple PQC modules. This finding proposes a key new step in the chaperone titration model that can explain why en masse shuttling of Hsp70 out of the nucleus is not needed to activate Hsf1. It remains unclear what cohort of defective proteins can recruit Sis1 and if this is the only mechanism for activating the HSR. Future work can elucidate how the authors' findings apply to regulation of human Hsf1 in health and disease.

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