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SPOTLIGHT

Fine-tuning stress responses by auxiliary feedback loops that sense damage repair

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Mogk and den Brave discuss exciting results from a comprehensive screen of heat shock response components in yeast, published in this issue by Pincus and colleagues (https://doi.org/10.1083/jcb.202401082). Their work reveals modulatory regulatory loops that fine-tune the timing of the shutdown of this highly conserved pathway.

Stress conditions cause imbalances of cellular homeostasis and trigger specific transcriptional responses to restore proteostasis. The evolutionarily conserved heat shock response (HSR) represents a prime example of the regulation of stress responses. The HSR is triggered by conditions that cause enhanced protein misfolding such as increased temperatures, as well as by ethanol, heavy metals, pathogens, inflammation, and cellular differentiation (1). In eukaryotes, the HSR is controlled by heat shock transcription factors (HSFs), which are activated upon stress application leading to increased expression of protein quality control (PQC) components, the heat shock proteins. The increased levels of PQC factors enable the repair or removal of damaged proteins, ultimately leading to the inactivation of HSFs during an attenuation phase. Six HSFs are encoded in the human genome with Hsf1 playing a dominant role in HSR regulation. Hsf1 activity declines during aging, and reduced Hsf1 activity is associated with neurodegenerative diseases including Alzheimer's and Parkinson's disease. On the other hand, high Hsf1 activity has been linked to specific cancers (2). Understanding how Hsf1 activity is tightly regulated has therefore high medical relevance and can guide therapeutic avenues for disease treatment.

Saccharomyces cerevisiae harbors Hsf1 as the sole and essential HSF, thus representing an excellent model system for studying HSR regulation. Previous work showed that both phases of the yeast HSR, initiation and attenuation, are controlled by the central Hsp70 chaperones. Hsp70 binds to Hsf1 in non-stressed cells and represses its activity (3). Stress conditions lead to the accumulation of Hsp70 substrates, causing depletion of free Hsp70 and leading to Hsf1 dissociation and activation (4). Enhanced expression of Hsp70 due to HSR activation initiates a negative feedback loop, which ultimately leads to the reinactivation of Hsf1 and prevents the overactivation of the HSR (5). It remained unclear whether the enhanced expression of other Hsf1 targets also contributes to HSR attenuation and whether their regulatory roles change depending on the particular stress conditions applied. This important point has been addressed by Pincus and colleagues in this issue of JCB with an excellent, systematic analysis of the yeast HSR (6).

The authors deleted the Hsf1-binding sites (HSEs) in the upstream activating sequences of 39 of the 42 Hsf1 target genes, disrupting their transcriptional induction upon heat shock. The impact of the HSE mutation on the HSR was monitored using a synthetic reporter, HSE-YFP, in which YFP expression is controlled by Hsf1. Determining YFP fluorescence during the time course of heat shock enabled the authors to study the roles of Hsf1 targets as negative feedback regulators. They identified six ΔHSE mutants exhibiting increased YFP fluorescence after 4 h of heat shock, implying contributions to HSR attenuation. Most of these ΔHSE mutants showed reduced growth rates at increased temperatures (37°C), suggesting defects in proteostasis. To visualize such defects, the authors employed the cellular disaggregase Hsp104-mKate as a fluorescent reporter for protein aggregates. Enhanced Hsp104-mKate foci formation in most ΔHSE mutants indicates enhanced protein aggregation and defects in aggregate clearance. Protein disaggregation by Hsp104 relies on cooperation with Hsp70, which targets the disaggregase to protein aggregates (7). This suggests a prolonged binding of Hsp70 to persistent cytosolic aggregates present in ΔHSE mutants and consequently a depletion of nuclear Hsp70 capacity. Indeed, such redistribution was observed for the major yeast Hsp70, Ssa1, and its crucial co-chaperone Sis1 upon heat shock in the ΔHSE-Fes1 mutant. These findings indicate that defects in Hsf1 inactivation are based on altered spatiotemporal regulation of Hsp70 in the diverse ΔHSE mutants. The functions of the identified feedback regulators in PQC can inform on their regulatory impact. Fes1 is a co-chaperone of Hsp70, catalyzing nucleotide release and substrate dissociation. Reduced

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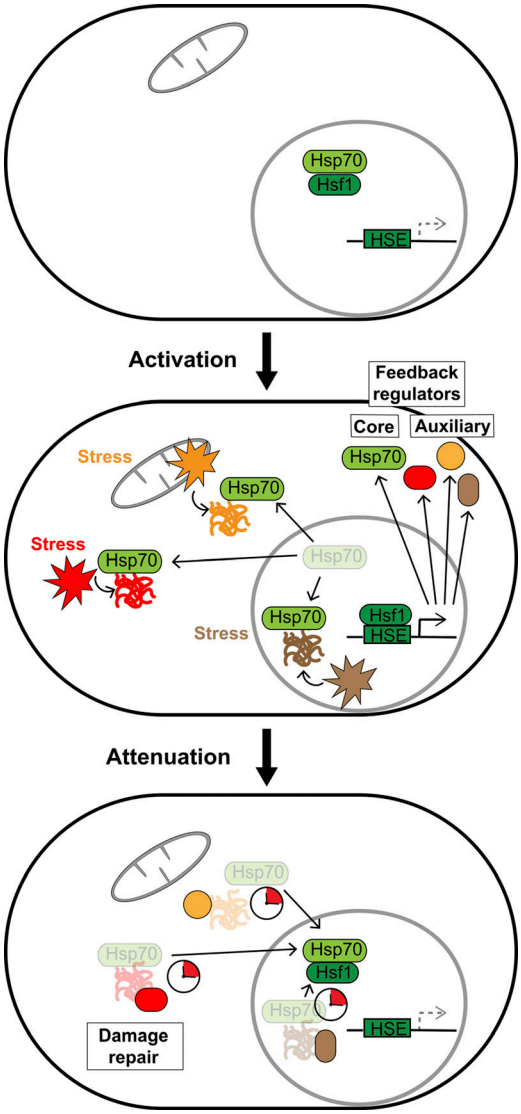

Figure 1. **Stress-specific feedback loops control the attenuation of the heat shock response (HSR).** Under basal conditions Hsp70 binds to Hsf1, preventing its binding to heat shock elements (HSEs). Activation: Diverse stress conditions cause protein misfolding and aggregation resulting in depletion of Hsp70 available for Hsf1 repression. Released Hsf1 binds to HSEs, triggering transcription of heat shock genes. These include the core feedback regulator Hsp70 as well as auxiliary factors. Attenuation: The diverse auxiliary feedback factors mediate and sense stress-specific repair. Thereby, they precisely adjust the timing of Hsp70 rebinding to Hsf1 and HSR inactivation.

levels of Fes1 in ΔHSE-Fes1 cells will slow down the operative cycle of Hsp70, thereby reducing the pool of Hsp70 available for Hsf1 repression. Ubi4 encodes concatemeric ubiquitin, while Gre3 detoxifies methylglyoxal, a reactive compound that can damage proteins. Reduced ubiquitin levels in ΔHSE-Ubi4 cells will weaken proteasomal degradation, while increased methylglyoxal levels in ΔHSE-Gre3 cells can cause enhanced protein damage. As a result, the burden for Hsp70 will be increased in both mutant cells, delaying protein repair and consequently Hsf1 inactivation.

To test whether the identified PQC components have a general or heat shock-specific function as negative feedback regulators, the authors repeated the entire analysis but with ethanol as an alternative stressor. Hsp70 remained the central and dominant feedback regulator upon ethanol treatment; however, a novel stress-specific set of Hsf1 targets was identified as feedback regulators that did not affect Hsf1 activity control during heat stress. These Hsf1 targets include the mitochondrial Hsp70 Ssc1 and its cochaperone Mdj1, pointing to proteostasis defects in mitochondria as a reason for prolonged Hsf1 activity in respective ΔHSE mutants. Such defects might lead to impaired import of mitochondrial precursor proteins, whose cytosolic accumulation has been shown to trigger an Hsf1-dependent stress response (8). Notably, the relative induction strengths of the individual Hsf1 targets remain unchanged upon ethanol stress (9), indicating that the initiation phase of the HSR is insensitive toward the specific stress regime applied, in contrast to the attenuation phase.

Together, these findings refine the mechanism of HSR attenuation, which is shaped by a two-tiered feedback architecture (Fig. 1). A core feedback loop is driven by enhanced expression of Hsp70 and is operative irrespective of the particular stress conditions applied. This loop reflects the central function of Hsp70 in PQC and links the general, Hsp70-dependent repair processes with Hsf1 control. This central loop is supported by stress-specific and variable auxiliary feedback loops for fine-tuning Hsf1 activity. The auxiliary loops precisely adjust Hsf1 target expression to stress-specific cellular injuries and limitations in damage repair. This regulatory principle has a far-reaching impact and will be applicable to other stress response pathways. The study also suggests that genetic variations in humans will lead to individual modulations of stress responses via the auxiliary feedback loops, potentially with disease-relevant outcomes.

The list of auxiliary feedback regulators is likely more extensive and their identification might require the combination of ΔHSE mutants to weaken compensatory activities. We also suggest that not necessarily the explicit cytosolic localization of Hsp70 but the mere persistence of protein damage represents the most crucial parameter of feedback control, irrespective of the cellular site. This should include the nucleus, which has been documented as an important PQC compartment (10). It will be also interesting to see whether and how ΔHSE and other mutants modify posttranslational modifications, which control the activity and stability of Hsf1. Such analysis might

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identify Hsp70-independent auxiliary feedback loops, leading to an even more sophisticated understanding of the HSR.

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