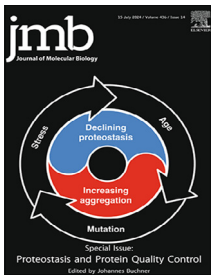
Review Article



The Heat Shock Response as a Condensate Cascade

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Abstract

The heat shock response (HSR) is a gene regulatory program controlling expression of molecular chaperones implicated in aging, cancer, and neurodegenerative disease. Long presumed to be activated by toxic protein aggregates, recent work suggests a new functional paradigm for the HSR in yeast. Rather than toxic aggregates, adaptive biomolecular condensates comprised of orphan ribosomal proteins (oRP) and stress granule components have been shown to be physiological chaperone clients. By titrating away the chaperones Sis1 and Hsp70 from the transcription factor Hsf1, these condensates activate the HSR. Upon release from Hsp70, Hsf1 forms spatially distinct transcriptional condensates that drive high expression of HSR genes. In this manner, the negative feedback loop controlling HSR activity – in which Hsf1 induces Hsp70 expression and Hsp70 represses Hsf1 activity – is embedded in the biophysics of the system. By analogy to phosphorylation cascades that transmit information via the dynamic activity of kinases, we propose that the HSR is organized as a condensate cascade that transmits information via the localized activity of molecular chaperones.

Discovery and biological relevance of the heat shock response

The origin story of the heat shock response has been told many times,[1–4] but it worth repeating to underscore how fundamental it is. The discovery of the heat shock response (HSR) dates to 1962 when Ferruccio Ritossa observed chromosomal puffing patterns in the salivary glands of *Drosophila*, suggesting a marked increase of transcriptional activity following exposure to elevated temperatures.[5] This was only a year after Jacob and Monod's seminal review describing the idea of gene induction.[6] From the initial description of the chromosomal puffs, to the connection between the puffs to the expression of heat shock proteins (HSPs), to the demonstration that heat shock proteins are molecular chaperones,[7,8] the HSR emerged as the best understood eukaryotic gene induction system. Elevated expression of HSPs during heat shock – i.e., the HSR – has been found to be conserved across flies, maize, yeast, mammals, and bacteria, suggesting deep evolutionary origins of the essential function of the HSR in cellular adaptation.[2,9]

The HSR has garnered sustained interest in multiple research communities. First, the HSR has established molecular precedents and provided general insight into how organisms are able to respond to environmental adversity beyond just

high temperatures.[10–12] The ability to adapt and maintain homeostasis in changing environments is crucial for organismal survival, and understanding the mechanisms for maintenance of cellular functions is fundamental to understanding physiology. Second, due to the rapid and robust induction of transcription of HSR target genes, the HSR has been a powerful model system for resolving the basic cellular processes controlling gene activation.[13–16] Third, by regulating the expression of molecular chaperones, the HSR plays a central role in protein folding and the broader protein homeostasis (proteostasis) network, and mis-regulation of the HSR has been implicated both in neurodegenerative diseases and cancer.[17–20] Thus, modulators of the HSR may have therapeutic potential in a wide range of human diseases. Finally, the HSR shows striking conservation in its molecular regulation across a wide range of eukaryotic organisms, enabling mechanistic dissection in model organisms.[2,9]

The early notions of the consequences of heat shock on cells was that the sudden increase in temperature resulted in proteotoxic damage that manifested in the formation of toxic aggregates comprised of partially denatured and misfolded proteins. In response to this damage, the HSR would be induced to help cells triage and degrade these aggregates. In this review, we synthesize data supporting an alternate model based on recent work: rather than toxic aggregates, the HSR is induced by "condition specific adaptive condensates" in yeast.

To define the terms, we use the word "aggregate" to denote an assembly of biomolecules without an adaptive role and "condensate" to refer to adaptive assemblies. While "aggregate" can be used generically to refer to any complex material formed from discrete components, and "condensate" can be used generically to refer to a dense material state of any form of matter, these terms have taken on connotations in biology that we make explicit here. Aggregate has a pejorative colloquial connotation due to the association of aggregates with neurodegenerative diseases like Alzheimer's and Parkinson's, so we will use it to describe assemblies like amyloid fibers composed of Aβ peptides, Lewy bodies containing α-synuclein, and amorphous agglomerations of unfolded luciferase used *in vitro* to study disaggregation. By contrast, "biomolecular condensate" was coined in 2017 as a function- and physical mechanism-agnostic catchall term to describe membrane-less organelles, dynamic signaling hubs, transcription factories, and any other non-toxic biomolecular assembly.[21] To emphasize the distinction between aggregates and condensates, we will typically include the adjectives "toxic" ahead of aggregate and "adaptive" ahead of condensate.

Hsf1 architecture, structure, and regulation by Hsp70

In eukaryotes, the HSR is controlled by transcriptional regulators known as Heat Shock Factors (HSFs). Here we will focus on the most conserved member of the family, Hsf1, and its regulation *primarily in budding yeast*. Reviews that focus on Hsf1 in human cells and other organisms can be found elsewhere.[22–24] Importantly for the context of this review, the evidence for the distinction between toxic aggregates and adaptive condensates – and the role of the latter in the HSR – is strong only in yeast. The extent to which the "condensate cascade" framework outlined below applies to cells in other organisms remains to be explored.

Hsf1 exists as a trimer and includes a core DNA binding domain (DBD) that recognizes a canonical motif known as the heat shock element (HSE) that functions as an enhancer in the upstream activating region of promoters of its target genes.[2,25] The winged helix-loop-helix DBD and leucine zipper trimerization domain (3mer) of Hsf1 are flanked on the N- and C-termini by intrinsically disordered regions (IDRs) that each contains a distinct Hsp70 binding site, the N-terminal element 1 (NE1) and the conserved element 2 (CE2) (Figure 1A). No structure of full length Hsf1 has been solved due to the large IDRs. Prediction of the trimer structure of the folded DBD-3mer core of Hsf1 (residues 147–424) using CollabFold[26] depicts the DBDs and leucine zipper with high confidence (Figure 1B, Video S1). Under non-heat shock conditions, Hsp70 is bound to Hsf1, maintaining the protein in its inactive state.[27–29] Upon heat shock, Hsp70 dissociates, allowing Hsf1 to bind to HSE-motifs, cluster into active transcriptional hubs, and activate its target regulon that includes molecular chaperone genes (Figure 1C).[4,30] Once cells have produced enough chaperones to restore homeostasis, Hsp70 binds to Hsf1 and inactivates the HSR, completing the negative feedback loop.[31]

This core Hsp70-mediated regulatory switch controlling Hsf1 activity is directly enforced by the J-domain protein and Hsp70 co-chaperone Sis1 and indirectly augmented by Hsf1 phosphorylation. Under basal conditions, Sis1 binds to free Hsf1 in the nucleus and transfers it to Hsp70 to repress the HSR.[32] Upon heat shock, Sis1 re-localizes from the nucleoplasm where it co-localizes with Hsf1 to the periphery of the nucleolus and to cytosolic foci where it is spatially separated from Hsf1, resulting in Hsp70 dissociation from Hsf1 and activation of the HSR. While Sis1 is required to repress Hsf1 under nonstress conditions, it is not a negative feedback regulator like Hsp70 because its transcriptional induction during heat shock is not required for deactivation of the

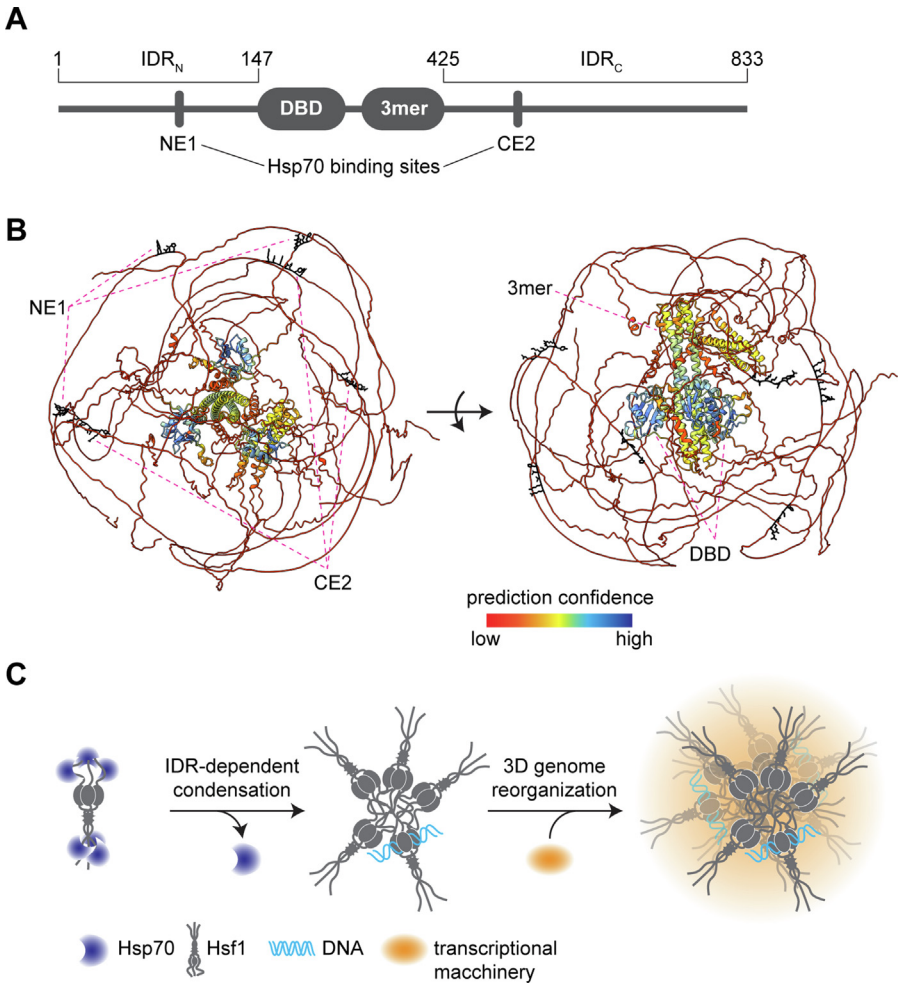


Figure 1. Structure and activation mechanism of budding yeast Hsf1. (A) Domain architecture of yeast Hsf1. IDR: intrinsically disordered region; NE1: N-terminal element 1; CE2: conserved element 2; DBD: DNA binding domain; 3mer: trimerization domain. **(B)** Structure of homo-trimeric Hsf1 as predicted by CollabFold, color coded by confidence score. **(C)** Schematic of the Hsf1 activation mechanism during acute heat shock, beginning with dissociation of Hsp70 and culminating in formation of intergenic transcriptional condensates.

HSR once homeostasis has been restored.[33] Hsf1 also becomes phosphorylated upon heat shock.[34] While phosphorylation is not strictly required for Hsf1 activation during heat shock, loss of phosphorylation results in lower HSR output during prolonged heat shock and reduced single cell variation in HSR output.[29,35] Conversely, mimicking constitutive phosphorylation results in constitutive Hsf1 activation.[29,36] These data suggest that phosphorylation operates in parallel to regulation of Hsf1 by Hsp70 and Sis1. Regulation of human HSF1 by Hsp70 binding and phosphorylation functions comparably.[37–39] At the mechanistic level, both chaperone binding and phosphorylation converge to regulate Hsf1 activity by dynamically controlling the formation and dissolution of transcriptionally active biomolecular condensates.[40,41]

Hsf1 transcriptional condensates

Transcriptional condensates are dynamic sites of active transcriptional activity that form within the nucleus of eukaryotic cells.[42,43] These hubs contain a high concentration of transcriptional machinery, including RNA Polymerase II (RNAPII), Mediator, and other transcription factors.[44,45] Formation of transcriptional condensates is driven by the multivalent interactions among IDRs found in transcription factors and RNAPII.[21,46] While originally described in mammalian cells, transcriptional condensates have now been observed in several instances in fungi, including the HSR.[30,40,47]

During acute heat shock, Hsf1 forms subnuclear clusters that colocalize with Mediator and RNA Polymerase II that serve as active sites of

transcription of the HSR genes. These clusters, termed HSR condensates, are internally dynamic, display rapid subnuclear reorganization, and are sensitive to 1,6-hexanediol, a reagent that disperses many biomolecular clusters associated via liquid–liquid phase separation.[30,48] Importantly, these active HSR condensates – which are conserved in yeast and human cells[30,41] – should not be conflated with the HSF1 condensates observed at satellite III repeats in human cells that do not regulate HSR genes and are rather early indicators of apoptosis.[49,50] For the HSR condensates in yeast, molecular genetic analyses revealed that the IDR_N region of Hsf1 is required for HSR condensate formation during heat shock, while the CE2 binding site for Hsp70 is required to prevent Hsf1 clustering under basal conditions. Beyond simply activating the HSR genes to high levels, the HSR condensates drive multiple HSR target genes – even those located on different chromosomes – to coalesce during heat shock.[30,40,51]

Transcriptional condensates would seem to confer several advantages for HSR activation. First, condensation could allow for a concentrated accumulation of transcriptional machinery to induce a robust HSR to restore homeostasis. Transcriptional condensates may also reduce molecular search time and enable coordinated activation across HSR genes separated by large genomic distances and distributed across chromosomes.[30] Spatial concentration of HSR genes thus allows for rapid, coordinated bursts for proper HSR activation. Surprisingly, however, these transcriptional considerations do not appear to be the primary selective advantage of HSR condensates. Separation-of-function mutants revealed that HSR condensate formation is dispensable for high level induction of the HSR target genes.[30] Even so, loss of HSR condensates still comes with a fitness cost at elevated temperature, indicating that the HSR condensates are playing an important role in the stress response.[30]

Since the biological relevance of the HSR transcriptional condensates cannot be explained by transcriptional output, it is likely that the condensates are playing a role in post-transcriptional gene control. The condensates could recruit mRNA modifying enzymes to mark the messages for privileged translation in the cytosol, or they could facilitate nuclear export of the induced mRNA molecules by interacting with the nuclear pore complex. Consistent with these post-transcriptional control mechanisms, HSR messages have been shown to bypass nuclear mRNA quality control and preferentially avoid translational repression during stress.[52,53]

Regardless of the molecular function of the HSR condensates, the self-association of Hsf1 into large, non-stoichiometric assemblies may provide an additional mechanistic regulatory layer. By forming condensates, Hsf1 may be performing a version of molecular mimicry, imitating the biochemical features and biophysical characteristics of the molecular clients that titrated the chaperones Sis1 and Hsp70 away from Hsf1 upon heat shock in the first place. In this manner, by making Hsf1 a more competitive substrate for Hsp70, condensate formation prioritizes the negative feedback loop to deactivate the HSR among the universe of clients Hsp70 could engage.

Toxic aggregates versus adaptive condensates

What are the molecular clients that titrate Sis1 and Hsp70 away from Hsf1? Precedent-setting studies demonstrated that protein aggregates form during heat shock and recruit molecular chaperones,[7,8] so it has traditionally been presumed that such protein aggregates activate the HSR.[2] The conceptual similarity of heat shock-induced aggregates to the protein aggregates found in post-mortem brain tissue from neurodegenerative disease patients, combined with the thermodynamically intuitive concept that increased temperature results in protein denaturation, seems to have provided the foundation for the longstanding assumption that a sudden increase in temperature activates the HSR due to the formation of toxic aggregates. In this toxic aggregate model, the proteome is thought to contain “metastable” proteins that are folded and functional under nonstress conditions but form heat-induced inactive aggregates. Metastable proteins can indeed be generated via mutations to endogenous proteins[54,55] or ectopically expressed from other organisms.[56,57] However, no endogenous yeast proteins have been reported to denature or aggregate at 37 °C. Since 37 °C robustly induces the HSR, metastable proteins are unlikely be the sole physiological ligands of the HSR.

Emerging to replace this model is a view in which the protein assemblies that form during heat shock are adaptive biomolecular condensates rather than toxic aggregates (Figure 2A). Heralding this paradigm shift, a major proteomic census of heat shock-induced protein sedimentation revealed that the fractions previously interpreted as proteotoxic aggregates contain active enzymes and are readily reversible, suggesting the aggregates are functional and reversible rather than toxic.[58] To underscore the point, there are currently at least four examples in the literature of adaptive heat shock-induced biomolecular condensates,[59–62] yet there remain no examples of heat-induced toxic aggregates. These data support a new understanding of the cellular response to heat shock in which adaptation begins with the spatial reorganization of the proteome into adaptive condensates prior to activation of the HSR, and these adaptive condensates may serve as the physiological ligands that

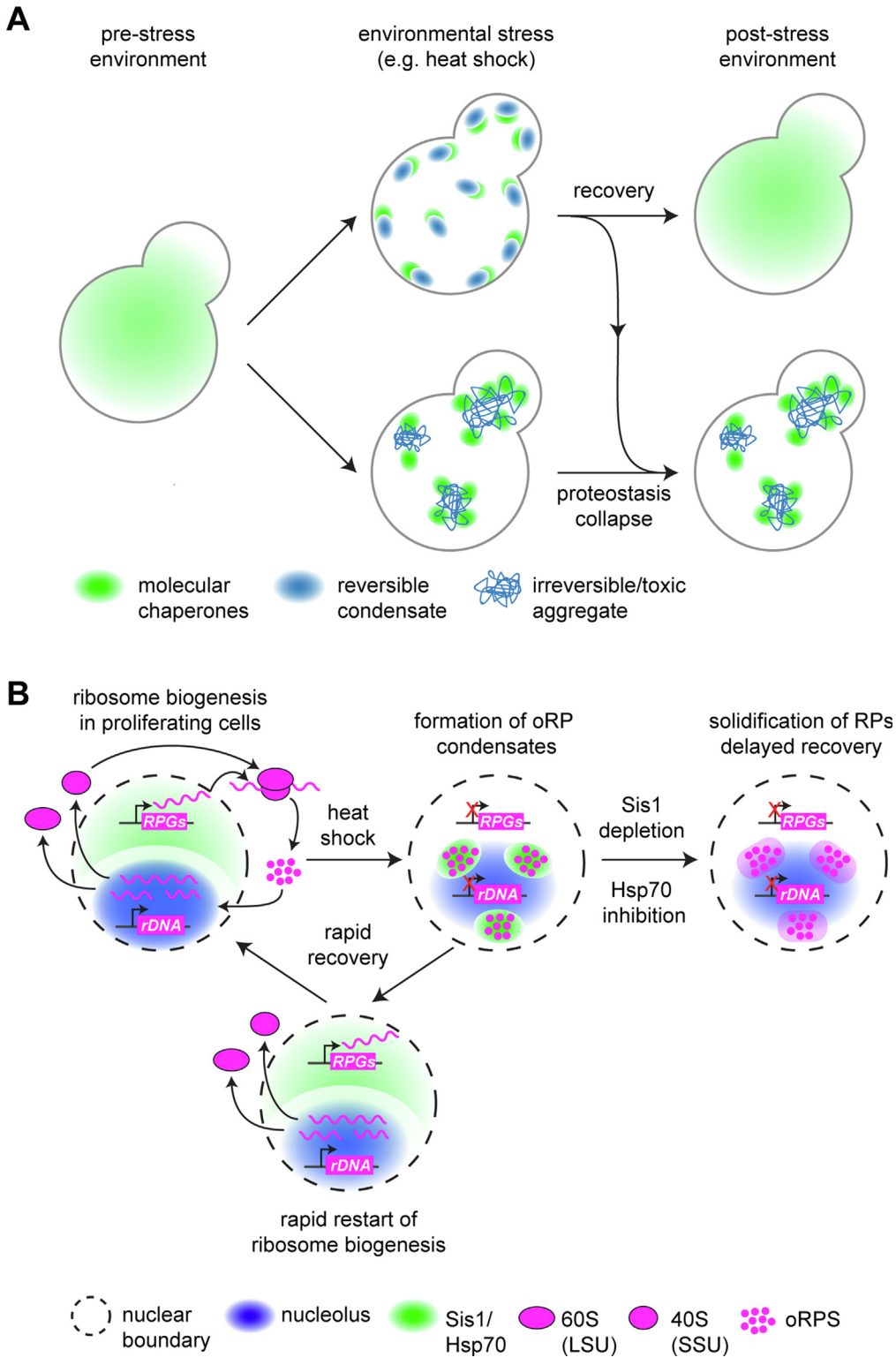


Figure 2. Formation and dispersal of adaptive stress-induced biomolecular condensates. (A) Stress-induced protein aggregation can be adaptive through the formation of reversible condensates or maladaptive through formation of irreversible and potentially toxic inclusions. Proteostasis collapse may result in the transition from reversible condensates to irreversible aggregates with loss-of-function and toxic gain-of-function properties. **(B)** Orphan ribosomal proteins (oRPs) form adaptive condensates at the periphery of the nucleolus upon heat shock that maintain their reversibility due to the activity of the chaperones Hsp70 and Sis1.

activate the HSR. Beyond the adaptive condensates identified so far during heat shock, different environmental cues may generate different condition specific adaptive condensates (CSACs) that activate the HSR.

Although no metastable endogenous proteins are known, studies expressing ectopic metastable proteins have revealed many insights into the spatial organization of the proteostasis network. These reporters have helped reveal emergent subcellular structures such as the insoluble protein deposit (IPOD) found in the cytosol, juxtanuclear quality control compartment (JUNQ), and intranuclear quality control compartment (INQ).[54,56,63,64] In addition, studies visualizing the subcellular organization of chaperone proteins during stress have revealed roles for the endoplasmic reticulum, vesicles, and the nucleolus in the cell biological response to heat shock.[32,65–67] The focus of many of these studies has been on protein degradation pathways operating in the cytosol and nucleus,[68–71] but evidence is mounting that the proteostasis network serves primarily to preserve proteins rather than degrade them during heat shock.

Orphan ribosomal protein condensates

Rather than metastable proteins that form toxic aggregates, multiple studies have implicated newly synthesized proteins and biomolecular condensates as primary activators of the HSR.[28,33,72,73] One set of proteins fulfills both criteria: orphan ribosomal proteins (oRPs).

During physiological heat shock, cells rapidly repress ribosome biogenesis during the acute phase of stress.[74,75] Eukaryotic cells are compartmentalized such that production of rRNA is housed in the nucleolus, while production of the ribosomal proteins occurs in the cytosol. Cells rapidly repress transcription of rRNA and ribosomal protein gene mRNAs in the presence of even mild environmental stress,[74,76] while translation remains active so that cells can mount a response.[58,61] Since ribosomal proteins cannot adopt their three-dimensional structures in the absence of rRNA, they are aggregation-prone and require specialized chaperones and nuclear import factors to remain soluble.[36,77] In actively dividing cells, nearly 50% of all ribosomes are translating ribosomal proteins at any given time, producing upwards of a million ribosomal proteins each minute.[78–80] Thus, upon heat shock, newly synthesized ribosomal proteins accumulate in large excess over rRNA and accumulate as oRPs at the nucleolar periphery.[62]

During the early events of heat shock, oRPs interact with Sis1, recruiting Sis1 to localize to the periphery of the nucleolus along with Hsp70 and form dynamic condensates. These condensates are liquid-like but depend on ATP and Hsp70 activity to remain so. Inhibition of Hsp70 or depletion of Sis1 leads to the solidification of oRPs, and transient depletion of Sis1 delayed cell growth upon recovery from heat shock. By maintaining oRPs in dynamic, liquid condensates, Sis1 and Hsp70 preserve the proteins in a functional state such that they can be readily incorporated into nascent ribosomes once cells resume rRNA synthesis[62] (Figure 2B). Thus, oRP condensates qualify as condition specific adaptive condensates (CSACs).

From the standpoint of the HSR, oRP condensate formation and recruitment of Sis1 to the nucleolar periphery occur in concert with Hsp70 dissociation from Hsf1 and formation of HSR transcriptional condensates in the nucleoplasm.[30,62] Importantly, preventing the accumulation of oRP condensates via conditional degradation of Ifh1, the master transcriptional regulator of ribosomal protein gene expression, resulted in diminished transcriptional output of the HSR.[62] In other words, oRPs drive HSR activation. However, oRPs can only account for a fraction of the total HSR output during heat shock, suggesting that many other ligands remain to be discovered.

Stress granules and other condition specific adaptive condensates

Aside from newly synthesized proteins like oRPs, the second major class of HSR agonists that have been proposed are CSACs comprised of mature proteins.[4,72] The best studied CSACs are cytosolic stress granules (SGs),[81] which are condensates that form at heat shock temperatures above 42 °C as well as in response to other environmental perturbations. SGs are comprised largely of mRNA and translation initiation factors and their composition is partly conserved from yeast to human cells. Purified SG components including Pab1 and Ded1 have been shown to autonomously phase separate with sharp temperature and pH boundaries, and for both proteins, condensate formation during stress is adaptive.[59,61] SGs are thought to privilege HSR transcripts for translation during stress by post-transcriptionally repressing non-HSR transcripts, and SGs are dispersed by chaperones induced by the HSR. During starvation, HSR gene transcripts escape condensation into SGs, allowing for privileged translation of Hsp70 and other chaperones,[52] and partitioning of Ded1 to SGs during heat shock biases translation toward mRNAs with low complexity 5′ untranslated regions, a set enriched for HSR gene transcripts.[61] Upon resolution of stress, SGs are dispersed via the activity of molecular chaperones, including Sis1, Hsp70, and the disaggregase Hsp104.[82]

In addition to operating downstream of HSR transcriptional activation as post-transcriptional regulators of HSR transcripts and physiological substrates for the chaperones those transcripts encode, SGs have been proposed to serve as

temperature sensors that condense to signal to augment the HSR at higher temperatures.[83] In this view, rather than unavoidable aggregation that signals chaos to the cell, the phase boundaries for condensation of SG components have been evolutionarily tuned to transmit information and activate the HSR precisely. While the list of CSACs is currently short – including just oRP condensates and SGs – and their link to the HSR is only via the single environmental condition of heat shock, we propose that additional CSACs exist in other conditions such as oxidative stress and starvation that activate the HSR (Figure 3A).

Condensate cascade model of the HSR

If CSACs are the upstream signals that activate Hsf1, and Hsf1 transcriptional condensates execute induction of the HSR genes, then HSR signaling can be said to proceed through a series of biomolecular condensates. We term this mode of signal transduction in which formation of one biomolecular condensate begets the formation of another a "condensate cascade." Analogous to how phosphorylation cascades transmit information through the dynamic activity of protein kinases, condensates cascades transmit information via dynamic subcellular localization their components. Moreover, since Hsf1 and many other proteins that form condensates are regulated by phosphorylation, classical phosphorylation cascades intersect with and regulate the properties of condensate cascades. In the case of the HSR, the cascade is mediated by the localized activity of Sis1 and Hsp70 that transmits the signal to activate the response.

Currently, only oRP condensates have been shown to be physiological ligands that initiate the HSR condensate cascade (Figure 3B). However, the modular architecture of the condensate cascade enables any other condensate that recruits Sis1 and Hsp70 to activate the HSR just as well. In *S. cerevisiae*, other environmental conditions trigger formation of distinct CSACs like SGs that may also activate the HSR (Figure 3C). Beyond *S. cerevisiae*, the condensate cascade could readily have evolved to take in other inputs. Consistent with this idea, in fungi that occupy hotter and colder ecological niches than *S. cerevisiae*, the temperatures that drive heat-induced condensate formation correspond precisely to the respective temperatures that activate the HSR.[84] While the HSR represents the founding example of a condensate cascade, we anticipate that other stress pathways like the unfolded protein responses in the endoplasmic reticulum and mitochondria may operate similarly given their regulatory parallels to the HSR.[85,86]

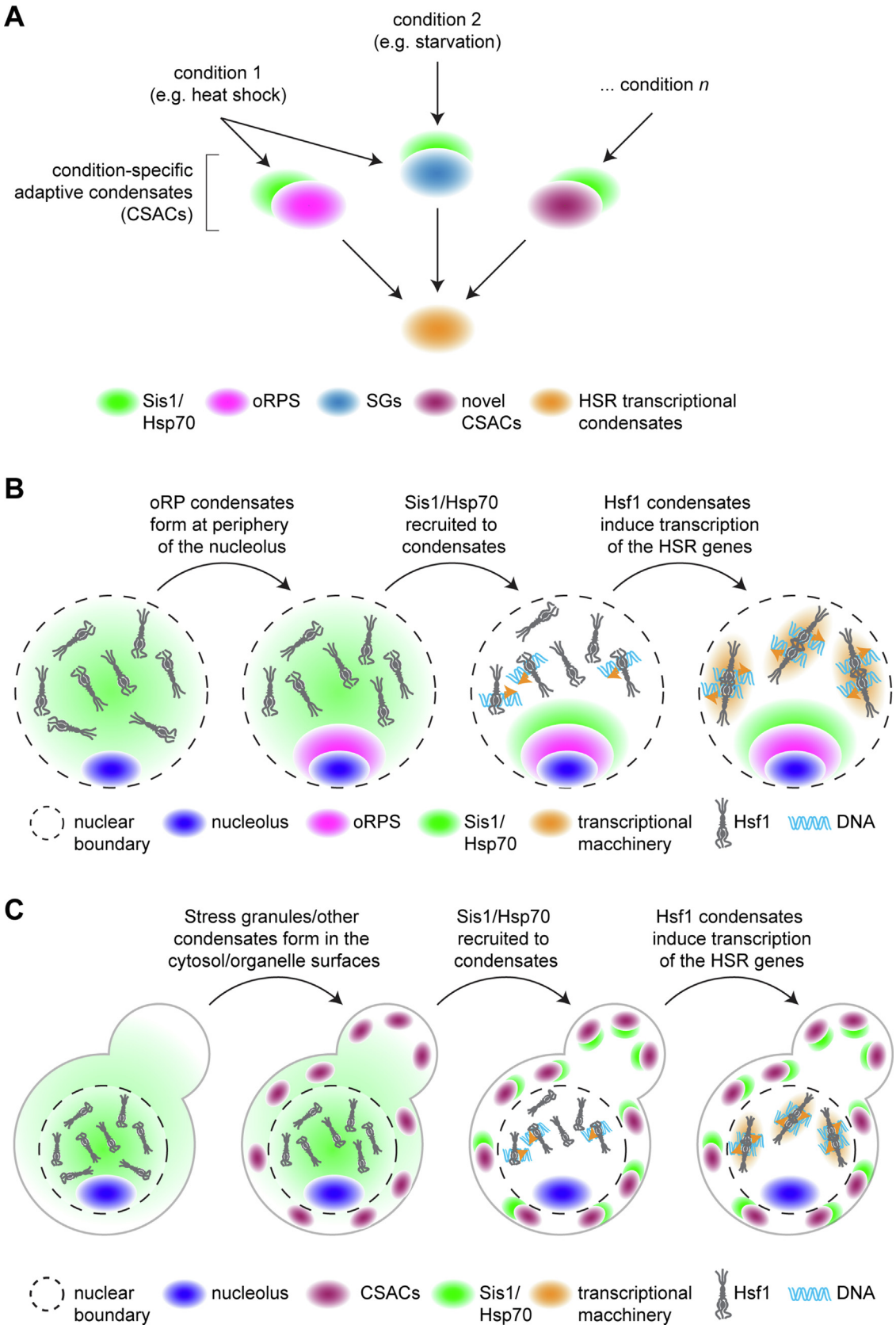
Implications in health and disease

Biomolecular condensates and the HSR are co-implicated in cancer, neurodegenerative diseases, and aging. For condensates, the mechanisms driving pathophysiology are diverse.[87–90] Genetic mutations can lead to changes in the multivalent interactions that drive phase transitions, impacting condensate formation, dispersal, and biophysical properties. In neuodegenerative diseases, mutations in the IDRs of several ribonucleoproteins results in a decrease in the threshold for liquid-to-solid phase transitions, leading to pathological fibril formation.[91,92] The TDP-43 and FUS proteins linked to Amyotrophic Lateral Sclerosis pathologies accumulate in SGs, leading to their depletion in the nucleus and loss-of-function consequences.[87] Extrinsic factors including stressful environmental conditions, altered activity of condensate regulators like chaperones or helicases, or a change in cellular metabolic state may also increase the propensity of maladaptive condensates to form.

Considering the relevance of phase separation in cellular function and pathophysiology, biomolecular condensates represent promising therapeutic targets.[93,94] As a guiding principle, it has been noted that liquid-to-solid phase transitions are generally associated with disease.[46,87] Moreover, there is evidence that the HSR responds much more robustly to the emergence of liquid-like condensates than solid aggregates associated with neurodegenerative disease.[95–98]

The link between mis-regulation of the HSR and aberrant condensate formation may be biophysically inextricable and a driver of disease spirals: in neurodegenerative disease, solid amyloids fail to activate the HSR, exacerbating

Figure 3. Condensate cascade model of the heat shock response. (A) Schematic of the HSR condensate cascade in which environmental conditions trigger the formation of condition specific adaptive condensates (CSACs) that titrate Sis1 and Hsp70 away from Hsf1, resulting in the formation of HSR transcriptional condensates. **(B)** Upon heat shock, oRPs form condensates on the surface of the nucleolus. The oRP condensates recruit Sis1 and Hsp70 away from the nucleoplasm where they were repressing Hsf1. Free Hsf1 then forms additional and distinct condensates with the transcriptional machinery to activate the HSR target genes. **(C)** Other stress-induced condensates like cytosolic stress granules, as well as unknown condensates that may form on the surface of organelles like the ER and mitochondria, may also initiate the condensate cascade to activate the HSR in response to other environmental inputs.


A
condition 2
(e.g. starvation)
condition 1
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condition-specific
adaptive condensates
(CSACs)
Sis1/
Hsp70
oRPS
SGs
novel
CSACs
HSR transcriptional
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proteostasis collapse; in cancer cells, aberrant condensates form that constitutively activate the HSR, supporting malignant growth. In the condensate cascade framework, altering condensate properties – either to "loosen" amyloids so that they may engage the HSR or to trigger solid aggregate formation and silence the HSR in cancer – may restore the adaptive capacity of HSR in a virtuous cycle.

Caveats and limitations of the model

The data currently supporting the condensate cascade model of the HSR are limited to a few studies primarily conducted in budding yeast, and we authored several of these. Indeed, oRP condensates are the only physiological ligand of the HSR currently known, and they have only been observed in a single condition so far—an abrupt switch from rapid growth at 30 °C to – 39 °C. The HSR is known to be activated in many other conditions, but the evidence that other CSACs like SGs also activate the HSR under these conditions remains circumstantial. As such, although the condensate cascade model has strong support in the context of activation of the yeast HSR at 39 °C, generalization beyond this precedent is speculation. The condensate cascade model of the HSR may be less applicable to terminally differentiated cells in multicellular organisms like human neurons that express many amyloidogenic proteins and in which ribosome biogenesis is relatively inactivate. More basic cell biology will be required in human cells in general and neurons in particular to determine whether the "rejuvenation" of solid-like aggregates we propose above will be a fruitful therapeutic avenue.

Outlook

Recent discoveries suggest that the HSR evolved not as a reactionary force to counteract toxic protein aggregates, but as a programmed network to manage adaptive biomolecular condensates. This model of the HSR as a condensate cascade opens new avenues for research into disease pathogenesis and therapeutic intervention. Targeting the condensate cascade offers a new approach to treatment that directly addresses the underlying biophysical disruptions. More broadly, the concept of biomolecular condensates as primary initiators of the HSR invites further exploration into the range of cellular conditions that converge on the cascade. Cataloguing the diversity of physiological ligands that trigger the HSR and other condensate cascades marks the horizon in unraveling cellular environmental response mechanisms.

CRediT authorship contribution statement

Annisa Dea: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **David Pincus:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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