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Coupling of Ribostasis and Proteostasis: Hsp70 Proteins in mRNA Metabolism

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Abstract

A key aspect of the control of gene expression is the differential rates of mRNA translation and degradation, including alterations due to extracellular inputs. Surprisingly, multiple examples now argue that Hsp70 protein chaperones and their associated Hsp40 partners modulate both mRNA degradation and translation. Hsp70 proteins affect mRNA metabolism by a variety of mechanisms including regulating nascent polypeptide chain folding, activating signal transduction pathways, promoting clearance of stress granules, and controlling mRNA degradation in an mRNA-specific manner. Taken together, these observations highlight the general principle that mRNA metabolism is coupled to the proteostatic state of the cell, often as assessed by the presence of unfolded or misfolded proteins.

Keywords

Hsp70; translation; mRNA decay; stress granules

The growing appreciation of Hsp70 protein function in post-transcriptional mRNA regulation

Control of the mRNA transcriptome is crucial for proper gene regulation. Within the nucleus, the mRNA transcriptome is controlled through modulation of transcription, splicing, and nuclear export. In the cytosol, mRNA function can be controlled through subcellular localization, as well as through changes in translation and decay. Certain stimuli and cellular states strongly regulate mRNA function through some or all of these mechanisms. In particular, stress responses dramatically alter the translation, degradation, and localization of mRNA [1}[2,3]). Finally, during stress the localization of mRNAs is controlled through accumulation of untranslated mRNAs either in stress granules, which are messenger ribonucleoprotein particle (mRNP) assemblies that are thought to contain

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mRNAs stalled in early phases of translation initiation [4], or alternatively in P-bodies, which are assemblies of untranslating mRNAs, translation repressors and components of the mRNA degradation machinery [5]. Since P-bodies and stress granules assemble in part through interactions between the prion domains or intrinsically disordered domains of RNA-binding proteins [6–9], proteins that modulate protein-protein interactions could be important in controlling mRNP granule assembly and/or disassembly.

A key set of proteins involved in protein folding and rearrangements of either aggregates or protein complexes are the Hsp70 proteins. Initially discovered as a class of 70KDa proteins induced during heat stress to deal with the increased load of misfolded proteins, it is now known that there are also constitutively expressed Hsp70 proteins [10,11]. Herein, we refer to both of these classes as Hsp70 proteins. Hsp70 proteins interact with numerous protein folding states (unfolded, misfolded, terminally aggregated) to promote protein refolding and/or to target misfolded proteins for degradation [12], and play roles in rearranging cellular protein assemblies. For example, Hsp70 proteins are known to play a role in the disassembly of clathrin coats [13] and in the disassembly/clearance of stress granules [14].

Hsp70 proteins typically function with Hsp40 proteins. Hsp40 proteins greatly increase the ATPase activity of Hsp70 proteins and also direct client protein specificity [12,15]. The ATPase cycle of Hsp70 proteins oscillates between the ATP-bound state (low substrate affinity) and ADP-bound state (high substrate affinity), which is promoted by Hsp40 proteins stimulating ATP hydrolysis by Hsp70. Importantly, different Hsp70:Hsp40 pairings elicit distinct outcomes for their client proteins [12], and therefore different Hsp40 proteins can provide specificity to the more generic Hsp70 proteins. Hsp70 function is also controlled by nucleotide exchange factors that promote the exchange of ADP for ATP, and thereby promote substrate release.

In the past 15 years, it has become increasingly clear that Hsp70 proteins play important roles in mRNA metabolism. In this review, we describe the diversity of mechanisms by which Hsp70 proteins impact mRNA metabolism. An important general principle is that by coupling mRNA translation or degradation to Hsp70 activity, it allows the cell to coordinate aspects of mRNA metabolism with the proteostatic state of the cell.

Effects of Hsp70/Hsp40 complexes on translation

Early evidence that Hsp70/Hsp40 proteins could affect translation came from three main types of observations. First, it was observed in yeast that conditional inactivation of Hsp70 or the Sis1 Hsp40 family member led to a decrease in translation as assessed by ^{35}S amino acid incorporation or polysome profiling [16,17]. Similarly, inhibition of Hsp70 function with chemical inhibitors or dominant negative alleles in mammalian cells also generally inhibited translation [18,19]. Second, Hsp70 and Hsp40 proteins are observed to associate with polysomes [17,19,20]. In yeast, there is even a dedicated Hsp70 protein, encoded by the Ssb1 and Ssb2 genes, that specifically interacts with ribosomes [20]. Finally, in yeast a conditional loss of function allele of Sis1 (an Hsp40 family member) is rescued by the same suppressor mutations that also rescue deletion of Pab1, a known promoter of translation

initiation [17]. From these results, it was inferred that Sis1 also has a role in translation initiation.

HSP70 enhances translation: nascent peptide folding

One way that Hsp70 proteins affect translation is by enhancing the folding of nascent peptides. This is suggested by the observations that Ssb1/2 (a ribosome-associated Hsp70 family in yeast) co-sediments with polysomes, interacts directly with nascent peptides [21,22], and that loss of Ssb function results in accumulation of aggregated nascent peptides [22]. Importantly, in higher eukaryotes the constitutive Hsp70 protein, referred to as Hsc70, is likely to perform this co-translational nascent peptide folding [23].

Two new studies demonstrate that the role of Hsp70 proteins in nascent chain folding is important for normal translation elongation [18,19]. The key result of these studies was that when either Hsp70 function was inhibited, or during proteotoxic stress, there was an increase in ribosomal pausing near in the first ~50 codons of ORFs [18,19]. The implication from these studies is that Hsp70 interacts with nascent peptides at the ribosome exit tunnel to promote translation elongation. During proteotoxic stress, Hsp70 would be titrated to other unfolded proteins, thus translation elongation is inhibited (Figure 1). The simplest model is that Hsp70 normally promotes elongation by binding to the nascent peptide and promoting its extrusion from the ribosome in a manner that promotes continued elongation. Consistent with this idea of protein:nascent peptide binding facilitating translation, an engineered heterologous protein interaction system enhanced translation only when the protein interacted with the nascent chain [19]. This supports a model of Hsp70-mediated nascent peptide ‘pulling’ from the ribosome exit tunnel to facilitate translation.

An unresolved issue is why ribosomes only accumulate near the beginning of the ORF when Hsp70 is inhibited. In principle, Hsp70 interaction with the nascent peptide could be important throughout the ORF. One possibility is that the initial N terminus of the protein is highly sensitive to Hsp70 loss since later in the ORF a more complete nascent peptide would be formed, which could have already initiated folding and thereby have interactions in *cis* that contribute to “pulling” the nascent peptide from the ribosome exit tunnel.

It should be noted that this model implies a hierarchical order of Hsp70 client proteins. That is, Hsp70 proteins preferentially act on misfolded or aggregated proteins that are present during stress, as opposed to nascent peptides associated with the ribosome. Whether this would be accomplished by extrinsic factors (e.g., a change in Hsp70 subcellular localization) or intrinsic factors (e.g., differential substrate specificity of constitutive vs. induced Hsp70 isoforms) is unknown.

HSP70 enhances translation: strengthening translation initiation mRNP

Some evidence suggests that Hsp70 function may also affect the function of specific translation factors, such as Pab1 and eIF4F. A connection of Hsp70 to general translation was implied since a lack of Hsp70 function in yeast resulted in a decrease in polysomes and ^{35}S incorporation [16]. A connection to Pab1 was proposed since Hsp70 defects led to a loss of the co-immunoprecipitation between Hsp70 proteins and Pab1 in heavy translation

complexes, which were interpreted to be polysomes [16]. This raises the possibility that yeast Hsp70 proteins also promote translation through maintaining Pab1 activity, which is known to enhance translation initiation.

Hsp70 has also been suggested to affect translation initiation through eIF4F, a translation initiation complex consisting of eIF4A, eIF4E, and eIF4G that binds the 5′ cap of mRNAs to recruit ribosomes in mammalian cells via an eIF4G:eIF3:40S interaction [26,27]. In addition, translation is further stimulated by a direct interaction between eIF4G and polyA binding protein (PABP) [28]. During prolonged heat stress in human cell culture, eIF4G becomes insoluble and associates less with eIF4E, which limits the availability of the critical eIF4F complex. However, this effect is reversed with simultaneous overexpression of Hsc70 [29]. One interpretation of these observations is that eIF4G is a relatively aggregation prone protein, and interactions with Hsp27, Hsc70 and other Hsps promotes its function by maintaining its solubility [29]. The greater implication is that Hsp70 proteins may be necessary for cellular processes that require components of differing solubilities (or aggregation propensities). Since there are multiple connections of Hsp70 proteins to translation, determining the impact of these Hsp70 interactions with translation initiation factors will require additional work.

Collectively, these results suggest multiple roles for Hsp70 and Hsp40 proteins in coupling translation to the proteostatic state of the cell, presumably as defined by competition for Hsp70s due to the pool of unfolded/misfolded nascent or mature polypeptides. One clear role is that Hsp70/Hsp40 function at the ribosome exit tunnel to fold/prevent aggregation of nascent peptides. A second, and more speculative, role is that Hsp70/40 proteins may regulate the folded state of key translation initiation factors and/or their interactions with each other.

Coupling of the transcriptome to ER stress: the Unfolded Protein Response

The population and function of the transcriptome is also directly coupled to proteostatic stress in the endoplasmic reticulum (ER) through the unfolded protein response (UPR), which is initiated in part by a HSP70 family member, referred to as BiP, localized to the lumen of the ER. When misfolded proteins accumulate in the ER, they titrate BiP away from sites on stress sensors, which triggers sensor activation [30,31]. For example, normally the protein kinase PERK dimerizes with BiP and is in an inactive confromation, but titration of BiP to unfolded ER proteins allows PERK to self-activate by dimerization and then downregulate translation in the cytosol by an inhibitory phosphorylation of eIF2α (Figure 1).

The proteostatic stress triggering the UPR response affects three key aspects of the transcriptome. First, in organisms from yeast to man, the Hac1/Xbp1 transcription factor is produced, which induces the transcription of genes to modulate the ER stress [32,33]. Second, at least in metazoans, PERK is activated by ER stress, and this leads to a broad and general downregulation of translation [34,35]. Finally, in Drosophila and mammals, ER stress activates the preferential degradation of mRNAs encoding proteins targeted to the ER, thereby downregulating the pool of newly incoming substrates for ER folding [36]. It is

striking how the sensing of misfolded proteins in the ER triggers such a broad response in mRNA metabolism to compensate for this perturbation of proteostasis.

Effects of cytoplasmic Hsp70/Hsp40 complexes on mRNA decay

Hsp70 proteins also have been suggested to play a role in mRNA decay pathways. Decay in eukaryotes is initiated by deadenylases, which remove 3′ adenosines from the polyA tracts of mRNAs. Once these polyA tails are at a critical length, 5′ decapping of mRNAs becomes kinetically favored, closely followed by 5′ to 3′ degradation by exonucleases, primarily XRN1. Alternatively, mRNAs can also be degraded via 3′ to 5′ decay either by the exosome, or (in mammalian cells) by a second 3′ to 5′ exonuclease called Dis3L [37–39].

One possible connection of Hsp proteins to mRNA decay is that stress can inhibit mRNA degradation in both yeast and mammals, mostly through inhibiting deadenylation [38]. Interestingly, it appears that this effect on mRNA decay rates in yeast requires a very extreme heat shock, since stabilization of mRNAs in *Saccharomyces cerevisiae* was observed at 46°C [3] but not 36°C [40]. This stabilization was attributed to an inhibition of the two major deadenylase complexes, the CCR4-NOT complex consisting of Ccr4, Pop2 and the Not proteins and the PAN2/3, which consisits of the catalytic Pan2 subunit and Pan3 [2]. Whether the effects of stress on mRNA decay involves Hsp70 function has not been directly examined, although there is no global change in mRNA decay rates in Hsp70 defective yeast strains at 37°C [41], which suggests that Hsp70 function may not be critical to this effect on mRNA stability.

Inhibition of ARE-mediated decay: possibly through direct Hsp70:RNA interactions

Several observations have suggested that Hsp70 proteins can directly modulate the decay rates of a subset of ARE-containing mRNAs. AREs are AU-rich sequence elements in the 3′ UTR of many mRNAs that typically promote rapid deadenylation and subsequent decay [42]. Evidence that Hsp70 proteins modulate mRNA decay stimulated by AREs first came from studies showing that heat shock stabilized reporter mRNAs with an ARE [43]. This effect appears to be due to Hsp70 proteins interacting with AUF1, a key RNA binding protein that binds ARE elements and promotes their mRNA degradation. Defects in Hsp70 function in yeast were also observed to lead to selective stabilization of the MFA2 mRNA in a manner dependent on an AU-rich mRNA decay element considered functionally similar to AREs [41,44]. In the case of the yeast MFA2 mRNA, Hsp70 function was required for normal deadenylation of the mRNA [41]. Both of these examples argue that Hsp70 can have a selective effect on differential mRNA decay rates, but the mechanism of this effect is not understood.

Some studies raise the possibility that Hsp70 may impact ARE-mediated decay by directly binding RNA. *In vitro*, Hsp70 binds RNA and prefers AU-rich sequences [45,46], U-rich sequences [47], polyA stretches [46], specific ARE sequences from individual mRNAs (TNFα; [49]), or an entire 3′UTR containing multiple AU rich elements (Bim mRNA/ Hsc70; [49]). Hsp70 binding to RNA is inhibited by ATP binding and is influenced by its peptide binding domain[45,6], arguing that RNA binding will be influenced by both substrate availability and the ATP state of Hsp70, which is influenced by Hsp40 and

nucleotide exchange factors. In addition to these studies, several Hsp70 proteins were independently identified as efficiently cross-linking to mRNA *in vivo* [46]. Interestingly, in this same study several mammalian Hsp40 proteins were also identified as possible RNA binding proteins, consistent with both Hsp70 and Hsp40 proteins interacting with mRNAs *in vivo*. A key experiment to determine if Hsp70 binding to mRNAs directly modulates specific mRNA function will be to map the Hsp70-mRNA interactions on a genome wide scale and determine the functional impact of those specific interactions.

Inhibition of ARE-mediated decay: alteration of effector mRNP

Some evidence suggests that Hsp70 proteins modulate the availability and/or composition of key protein complexes that affect mRNA turnover rates. ARE-containing mRNAs are stabilized during heat shock [43], where Hsp70 expression is induced. It is thought that AUF1 binds to PABP, either removing it from the polyA tail, and/or recruiting deadenylase components, thus initiating decay [48]. Hsp70 may inhibit ARE-mediated decay as it disrupts the AUF1:PABP interaction *in vitro* [44], and is found in a complex with AUF1 and PABP with heat stress *in vivo* [43]. However, *in vitro*, it is principally the addition of an ARE-containing RNA that inhibits the AUF1:PABP interaction [48]. The presence of ARE-containing mRNAs in Hsp70:AUF1 complexes was not examined *in vivo* [43].

There is an additional example of Hsp70 proteins changing mRNP complexes in mammalian cells. During steady state conditions, a pro-apoptotic ARE-containing mRNA, Bim, is bound by Hsc70 via its 3′UTR [49]. With interleukin-3 treatment, the Bim mRNA is destabilized, concurrent with loss of Hsc70 binding to the mRNA and the formation of an Hsc70, Bag4 (a nucleotide exchange factor), and CHIP (an E3 ubiquitin ligase) complex [49]. In this manner, Hsc70 plays a role in stabilizing the mRNA and its removal may allow for the binding of other proteins that stimulate mRNA turnover.

Taken together, it is now clear that Hsp70 function can affect mRNP composition and the degradation of at least some mRNAs.

Hsp70/Hsp40, translational recovery from stress and clearance of stress granules

Several pieces of data argue that Hsp70/Hsp40 complexes play a role in helping translation recover from stress. First, knockdown of Hsp72 in mammalian cells prevented translation recovery after prolonged proteasomal inhibition [51]. Second, in Drosophila S2 cells, adding a pan-Hsp70 inhibitor during recovery from heat stress limited the reformation of polysomes [52]. Third, in human cell culture, Hsp70 overexpression rescues this translational repression caused by overexpression of the prion related domain (PRD) of the stress granule component TIA-1, a sequence specific RNA binding proteins [6]. Finally, yeast strains lacking the Ydj1 Hsp40 protein were shown to be compromised in translation recovery after NaN_3 stress [14].

Additional evidence suggests that yeast Hsp70/Hsp40 complexes play roles in the disassembly and/or clearance of stress granules. Not only do stress granules contain Hsp70

and Hsp40 proteins [14], but stress granule clearance is reduced during stress recovery in both Hsp70 and Hsp40 deficient yeast strains [14].

An unresolved question is how stress granule dissipation directly relates to translation recovery. It appears clear that stress granule formation and translation are inversely correlated [4]. Moreover, stress granules contain mRNPs that resemble stalled translation complexes [53,54], and often loss of stress granules coincides with resumption of translation [14,51,55]. However, there are at least two situations where cells resume translation while maintaining stress granules [14,56]. One explanation is that there may be subclasses of stress granules, or components within individual stress granules, that have distinct fates: some will remodel to re-enter translation, and some will be trafficked to the vacuole for degradation [14].

Some evidence now argues that either different stress granule components, or different classes of stress granules, have different fates during stress granule recovery. First, during recovery from paraquat-induced stress granules in mammalian cells, HuR exits stress granules efficiently while TDP-43 remains present in granules for prolonged periods [58]. Second, while deletion of the yeast Hsp40 family members Ydj1 and Sis1 similarly caused stress granule persistence after stress, translation recovered in Sis1-depleted but not Ydj1-depleted cells after stress [14]. These results have been interpreted to suggest that Ydj1 affects stress granule disassembly in a manner that promotes mRNAs re-entering translation, while Sis1 targets them for degradation in the vacuole (Figure 2, Key Figure). Thus, subsets of stress granules, or their components, have alternative fates based on the function of specific Hsp40 proteins.

Collectively, these observations suggest that, at least in some circumstances, Hsp70s are necessary for translation recovery and stress granule dissipation.

Concluding remarks

The observations discussed in this review highlight the multiple mechanisms by which cells couple the proteostatic state of the cell to modulate mRNA metabolism through Hsp70 family members. Hsp70 proteins can directly affect translation by regulating nascent polypeptide chain folding, which can feedback on translation elongation. At least in the lumen of the ER, an Hsp70 family member functions to limit a proteostatic stress signal transduction pathway. This example illustrates the principle that Hsp70 family members may play a broader role in controlling cell signaling in response to proteostatic stress. Finally, Hsp70 proteins have been described to affect both individual mRNP composition, as well as macromolecular mRNP stress granules. Important areas of future work will be to determine the specific mechanisms of these effects, and if other regulatory systems coupling proteostasis and ribostasis exist.

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OUTSTANDING QUESTIONS

Several significant questions about how proteostasis and ribostasis are coupled to allow optimal cellular function.

1. How do changes in proteostatic stress perturb Hsp70 action on specific client proteins and do cells use this mechanism to modulate key aspects of mRNA biogenesis and function during stress conditions?
2. What is the global nature and scope of Hsp70 RNA binding? A global analysis of Hsp70 RNA binding may yield important insights about the specificity and function of direct Hsp70:RNA interactions.
3. Are the functions of Hsp70/40 in translation recovery and stress granule clearance directly linked? A related issue is what the targets of Hsp70 activity within stress granules are. One intriguing possibility is that the high degree of intrinsically disordered protein domains in these assemblies creates a high local context of Hsp70/Hsp40 clients, which is important to disassemble to allow recovery from stress.
4. Given the importance of cellular homeostasis, are there other regulatory pathways that ensure that proteostasis and ribostasis are properly balanced?

TRENDS BOX

- The proper control of the proteome (proteostasis) and transcriptome (ribostasis) is critical to cell function.
- By multiple mechanisms, Hsp70 family members sense perturbation of proteostasis and then modulate aspects of mRNA metabolism.
- Hsp70 family members promote nascent protein folding and when defective this leads to an inhibition of translation elongation.
- Hsp70 family members can be titrated by unfolded proteins leading to the activation of stress responsive signal transduction systems that modulate the transcriptome.
- Hsp70 family members can modulate the protein composition of individual mRNPs, thereby affecting their function.
- Hsp70 proteins promote disassembly of stress granules and are important for recovery of translation after stress.

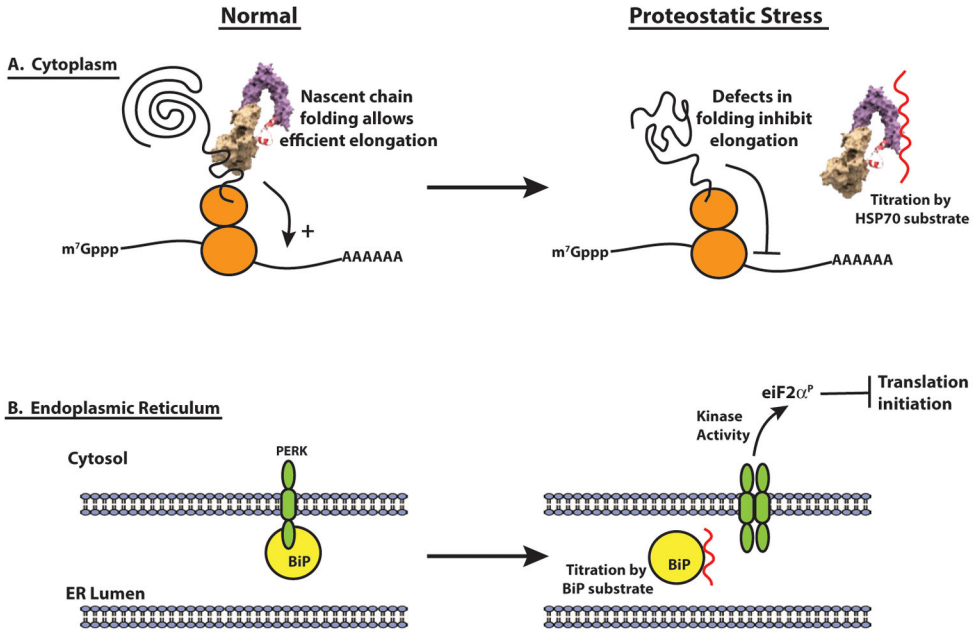

Figure 1.

Hsp70 effects on translation changes with stress. In the cytoplasm (A), Hsp70 proteins bind nascent polypeptides, thus enhancing translation. However, when cells are under proteostatic stress, Hsp70 no longer interacts with nascent peptides, which decreases translation elongation. In the endoplasmic reticulum (ER) (B), the Hsp70 family member Binding Immunoglobulin protein (BiP) normally binds PRKR-like endoplasmic reticulum kinase (PERK) in the ER. Misfolded/unfolded proteins in the ER titrate BIP away from PERK, allowing PERK activation, phosphorylation of eukaryotic initiation factor 2α, eIF2α and global downregulation of translation initiation.

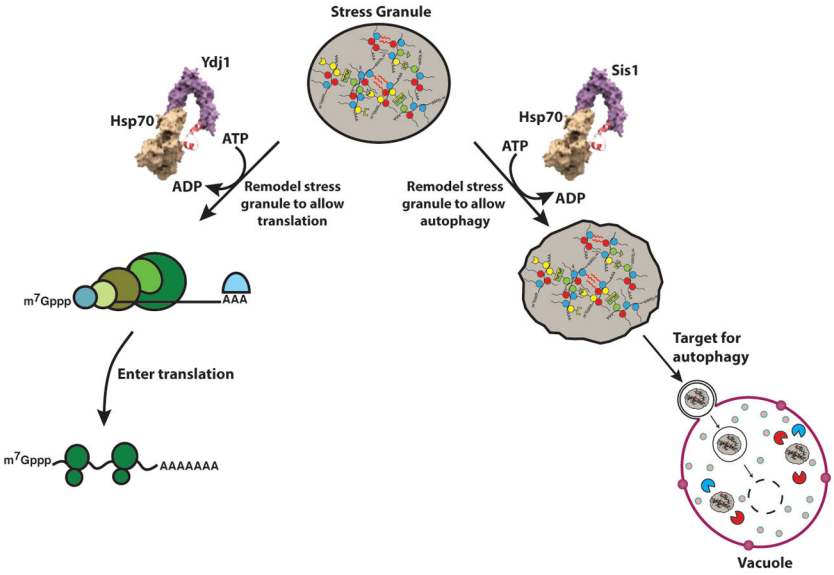

Figure 2. Key Figure

Distinct Hsp70:Hsp40 pairings elicit different stress granule outcomes. Hsp40 member Ydj1 may resolve stress granules to facilitate translation recovery following stress (left). Conversely, Hsp40 Sis1 traffics stress granules to the vacuole for degradation.