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Cell adaptation upon stress: the emerging role of membrane-less compartments

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Cells under stress transition from a growth to a quiescent state. The conventional thinking is that this is achieved through transcriptional programs, translational regulation, protein degradation, and post-translational modifications. However, there is an increasing realization that stress adaptation also goes along with dramatic changes in the architecture and organization of cells. In particular, it seems to involve the formation of membrane-less compartments and macromolecular assemblies. We propose that cells make widespread use of this ability to change macromolecular organization to adapt to stress conditions and protect themselves.

Here, we address what triggers the formation of these assemblies under stress conditions. We present examples illustrating that in some cases, sophisticated signaling pathways transmit environmental fluctuations from the outside to the inside and in others, that external fluctuations directly affect the internal conditions in cells. We further argue that changes in the organization of the cytoplasm and the formation of membrane-less compartments have many advantages over other ways of altering protein function, such as protein degradation, translation or transcription. Furthermore, membrane-less compartments may act as protective devices for key cellular components.

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Introduction: adaptation to stress

Eukaryotic cell organization is sustained by numerous membrane-bound organelles, but also by membrane-less compartments (such as the nucleolus and P-bodies) that all acquire and maintain a unique functional identity. How this is achieved under steady state conditions is beginning to be understood, but a clear picture of how cells adapt to external perturbations and environmental stress while keeping most of their integrity is still lacking. This is especially complex given that stress inhibits many of the metabolic pathways.

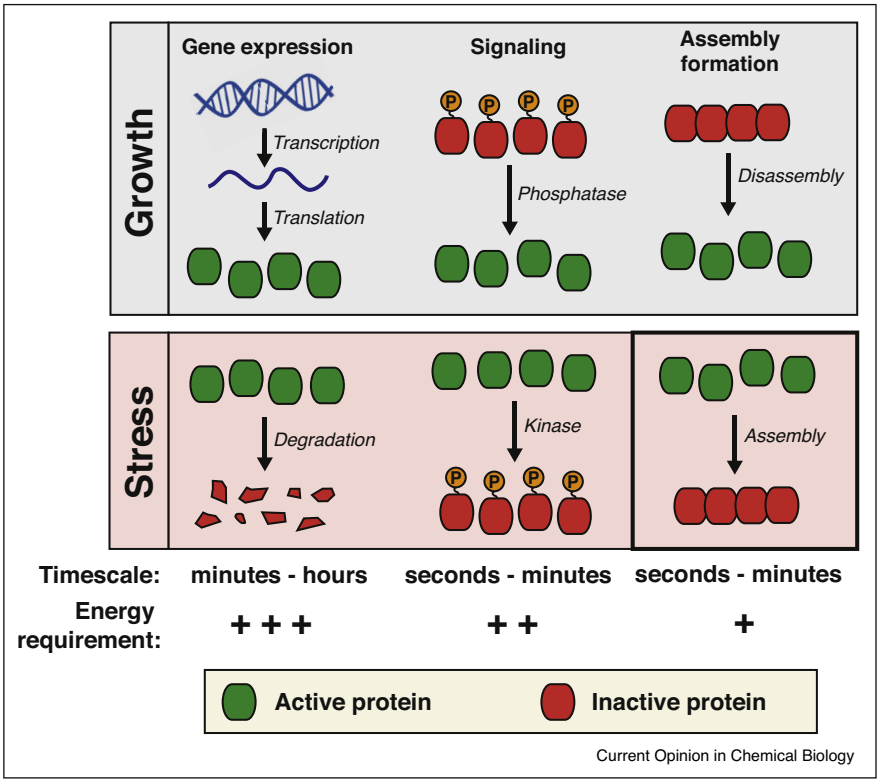
Stressed cells transition from a growth state to a quiescent state. They leave the cell cycle, down-regulate housekeeping functions, change their metabolism and upregulate stress-protective pathways. The conventional view is that this is achieved through the activation of signaling pathways, leading to post-translational modifications of proteins that are turned on or off, as well as modulation of gene expression, or a combination of both [1,2] (Figure 1). In this regard, the best understood stress response is that to heat stress. Heat shock leads to widespread protein misfolding and aggregation. This elicits an upregulation of so-called Heat Shock Proteins (Hsp), which help in preventing aberrant behavior of misfolded proteins that tend to aggregate and form microscopically visible structures [2,3].

However, not all cellular structures that form under stress are a result of uncontrolled protein misfolding and aggregation. Indeed, increasing evidence shows that many stress-inducible membrane-less structures form in a highly regulated manner and incorporate proteins in their (near) native conformations [4–7,8••] (Figure 1). In this review, we call such protective structures stress assemblies to differentiate them from protein aggregates.

Stress assemblies contain many different proteins but also often RNAs. These assemblies range in size from several hundred nanometers to micrometers, meaning that they contain hundreds to millions of macromolecules. They also have specific properties and functions, and may act as storage and release depots for macromolecules, as well as micro-reactors for concentrated biochemistry [5••,8••,9–11].

The first described stress assemblies were heat stress granules in plant cells [12–14] that belong to the larger category of stress granules [15,16]. They form as a result of protein translation inhibition/stalling, leading to the accumulation of mRNAs that are bound to RNA-binding proteins (such as FMR1, TIA-1, G3BP, Caprin), translation factors and 40S ribosomes [17]. Since then, systematic studies with stressed cells suggested that many such

Figure 1



Different modalities of stress adaptation.
Cells generally use three different mechanisms to adapt to stress. They can change the expression of a protein; they can regulate the activities of the protein through post-translational modifications; or they can form assemblies to sequester the protein (black box).

stress assemblies exist [4,18,19]: For instance, glutamine synthase [4,8••] and CTP synthase filaments [6] form in starved yeast as well as in *Drosophila* [20]. In mammalian cells, purinosomes are structures that form as a response to serum starvation [21]. And the list of stress assemblies continues to grow. Two assemblies that have recently been added are the Sec bodies in *Drosophila* [22••] and the A body in human cells [23••]. Interestingly, the material properties of these assemblies are very diverse: some assemblies behave as solid-like crystals, glasses or gels, whereas others are extremely dynamic and show liquid-like properties [11,24,25•]. Differences in the material properties can be determined by various biophysical methods that measure the shape or dynamics of these assemblies, such as fluorescence after photobleaching (see below).

Here, we focus on emerging links between metabolic stress and stress assemblies, in budding yeast and in *Drosophila*. We discuss what triggers the formation of stress assemblies: are there sophisticated signaling pathways that transmit environmental fluctuations from the outside to the inside, or do external fluctuations directly affect the internal conditions of cells? In the two examples we report below, we provide evidence for both mechanisms.

Yeast adapt to starvation by changing the physical properties of their cytoplasm

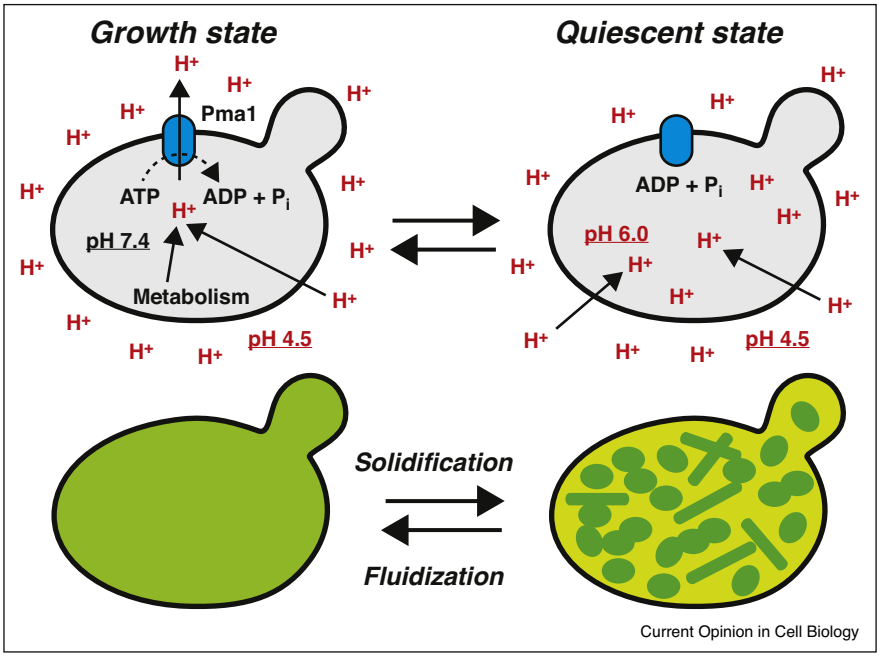
The first example is the response of budding yeast to starvation, which illustrates how external fluctuations directly affect the internal conditions in cells and trigger the formation of stress assemblies that are protective and pro-survival.

Stress and starvation cause a drop in intracellular energy levels

A strong link between energy levels and stress has been established [26,27••]. Indeed, maintenance of the normal state of a growing cell requires constant supply and consumption of energy through synthesis and hydrolysis of ATP. This energy fuels metabolism and is used to keep key parameters, such as the intracellular ion concentration, the osmotic pressure and the cytosolic pH, in a range that is favorable to growth (Figure 2).

However, for cells that are exposed to sudden stress, the metabolism comes to a standstill, making it difficult to

Figure 2



Starved yeast cells switch between a growth (left) and a quiescent (right) state.
Budding yeast cells enter into a quiescent state upon removal of nutrient. This causes a drop in cytosolic pH from 7.4 down to around 6.0, because starved yeast cells lack the energy to maintain the pH gradient across their plasma membrane. The intracellular increase in proton concentration triggers a phase transition of the cytoplasm from fluid to solid-like. When conditions improve, yeast fluidize their cytoplasm again by neutralizing the pH. Pma1 is a proton pump that carries protons from the inside to the outside, using up ATP in the process.

maintain ATP levels. Consequently, stressed cells undergo extreme fluctuations in ion concentration, osmotic conditions and pH levels. These fluctuations are likely to change the solubility and interactions of macromolecules, leading to the formation of stress-adaptive assemblies. We recently postulated that assembly formation in response to fluctuations in internal conditions is a simple and effective way of regulating cell function in situations of cellular crisis [5••,8••].

Assembly formation during stress changes the properties of the cytoplasm

Consistent with this idea, a multitude of proteins have been shown to form structures when yeast cells are depleted of energy [4,5••,8••]. Some of these structures are highly ordered, as shown by the example of metabolic enzymes that assemble into polymers or crystals in starving cells [6,8••]. Other assemblies are more irregular and heterogeneous. The material properties of these heterogeneous assemblies are still unclear, but they most likely are multi-component gels or glasses [4,5••]. Our recent findings show that the widespread formation of such structures under stress conditions also changes the physical properties of the cytoplasm: it transitions from a fluid to a solid-like state [5••]. This makes the stressed yeast cells so stiff that they can keep their shape, even in the absence of a cell wall. Although there is some debate as to whether these higher-order structures are functional [11,28], we favor the view that many of those are adaptive, because they allow stressed cells to exit the cell cycle, downregulate metabolic pathways or protect macromolecules.

Drop in cytosolic pH induces solidification of the cytoplasm

But how do yeast cells achieve such a massive change in the properties of the cytoplasm? Solidification of the cytoplasm seems to be induced by a fast (few minutes) decrease in cytosolic proton concentration (which spans almost two orders of magnitude) triggered by energy depletion [5••] (Figure 2). Such extreme fluctuations in proton concentration may affect many protein-protein interactions, presumably by remodeling the surface charges of these proteins, especially those with a low isoelectric point, thus leading to widespread changes in the organization and properties of the cytoplasm [5••]. This is further accelerated by a concomitant decrease of cell size and increased macromolecular crowding [29••].

Is cytoplasmic solidification also operational in dormancy?

Our findings with energy-depleted yeast suggest a link to a widespread phenomenon in nature called dormancy [30]. Dormancy is defined as an extended period of

inactivity and reduced metabolic rate, and it often goes along with the formation of specialized cell types, such as spores or seeds. A dormant state is frequently induced through adverse conditions, such as a lack of nutrients or water. The molecular mechanisms of dormancy are still very poorly understood, but the cytoplasm of dormant spores or seeds is in a gel-like or glass-like state [31–33] that is reminiscent of the solid-like state observed during acute starvation in yeast [5••] or bacteria [34]. A functional connection is further supported by the finding that many stress assemblies are also found in yeast spores [5••]. Thus, solidification of the cytoplasm could be a frequently used means to downregulate metabolism under conditions of acute stress but also under conditions of developmentally controlled dormancy.

Assembly formation is reversible and promotes cellular survival

Assembly formation under stress conditions is only useful when the formed assemblies can be reversed and the sequestered proteins are not damaged in the process. For starved yeast this may involve a rebounding of the cytosolic pH [5••] and an increase in cell size [29••]. As a result, the solid yeast cytoplasm is quickly reverted into a fluid state. Importantly, when energy-depleted yeast cells are prevented to enter into a solid-like state, they die. This indicates that the solid-like state of the cytoplasm is functionally important, presumably because it allows starved yeast cells to stop diffusive processes in the cytoplasm and downregulate metabolism.

The speed by which a yeast cell enters and exits the solid-like state (minutes rather than hours) suggests that this process is neither mediated by signaling nor changes in gene expression. Taken together, our findings indicate that fluctuations in key internal parameters can directly change the properties of the cytoplasm on a local and global scale. But are there other ways to form stress assemblies?

Sec bodies form in *Drosophila* upon amino-acid starvation

The second example we discuss reports the stimulation of a specific signaling pathway that transmits environmental fluctuations from the outside to the inside of the cell.

As eluded above, cellular stress often leads to the inhibition/stalling of anabolic pathways, such as protein translation, which leads to stress granule formation. A number of known signaling pathways trigger their formation, all dealing with translation arrest, such as elF2alpha phosphorylation. Additional evidences suggest that phosphorylation by DYRK kinases and glycosylation play a role in stress granule dynamics [35,36]. However, only few proteins have been identified that assemble into stress granules or other stress assemblies upon signaling and post-translational modifications.

Sec bodies are induced by amino-acid starvation

Light on post-translational modifications that play a role in stress assembly formation has been shed by the study of Sec bodies. They form in *Drosophila* cells in response to the inhibition of another anabolic pathway, the protein transport through the secretory pathway, induced by amino-acid starvation [22••].

The secretory pathway comprises multiple discrete membrane-bound compartments that communicate with one another via small vesicular carriers. One of these carriers are COPII-coated vesicles that are formed upon the recruitment of COPII subunits and the large scaffold protein Sec16 [37••] to the membrane at ER exit sites. Upon amino-acid starvation, COPII subunits, Sec16 and many other proteins that remain to be identified are incorporated into membrane-less stress assemblies, the Sec bodies (Figure 3). Sec bodies form specifically upon amino acid starvation in a time frame of hours rather than minutes.

Sec body material properties: liquid, not solid

Contrary to the solid-like properties of some of the macromolecular assemblies described in energy-deprived yeast, *Drosophila* Sec bodies appear to have many of the properties normally attributed to liquid droplets. In addition to being round, they show rapid diffusive behavior (as measured by fluorescence recovery after photobleaching, FRAP). When half of a Sec body marked by Sec16-GFP and Sec24-GFP is photobleached, the fluorescence recovery is very quick because molecules within move very rapidly from the bleached side to the non-bleached [22••]. These FRAP properties suggest that Sec bodies are liquid-like droplets.

Sec bodies promote survival and can be reversed

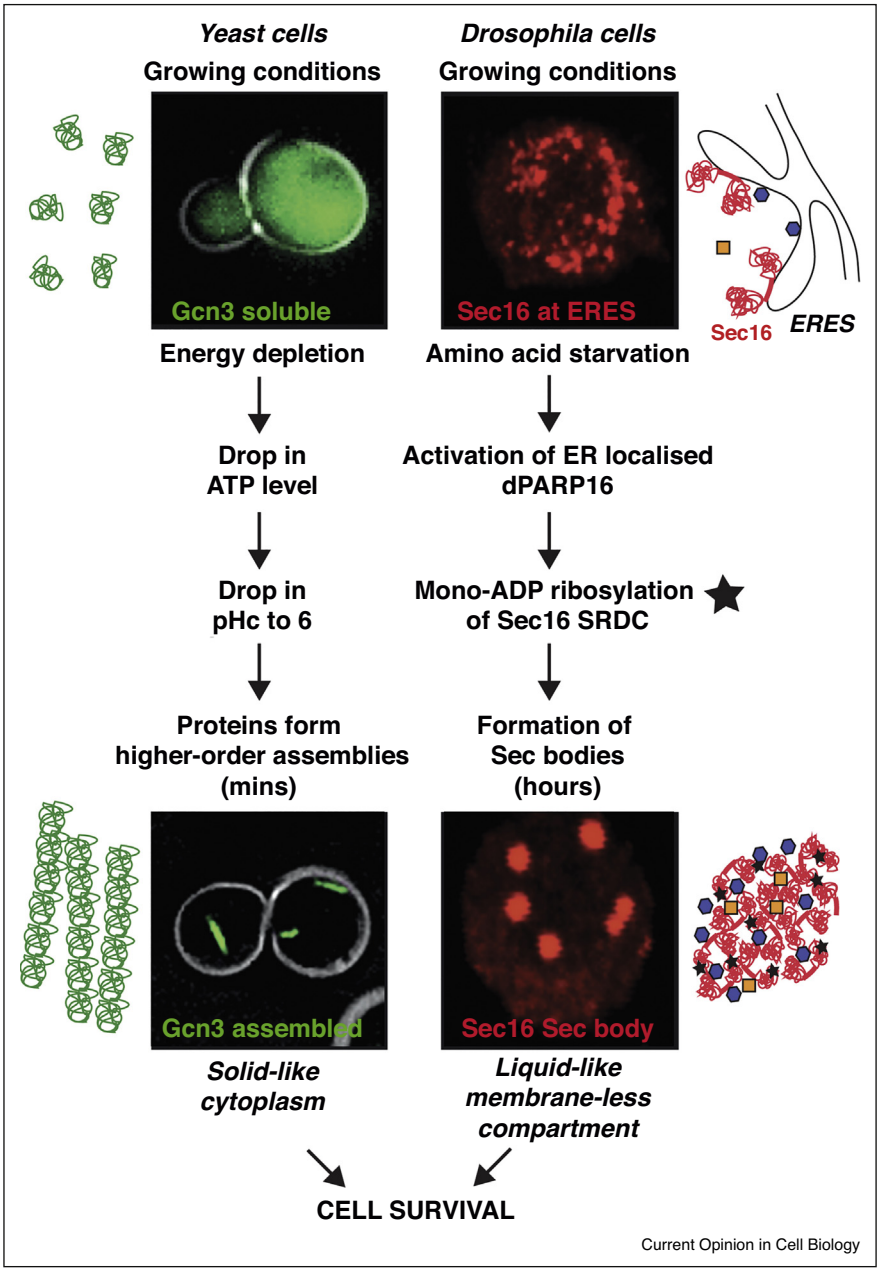
As the starved yeast stress assemblies, Sec bodies are rapidly reversible upon replenishing the medium with amino-acids. They act as a reservoir for COPII subunits and Sec16 that are re-used to sustain ER exit upon re-feeding. This conclusively shows that the proteins contained in Sec bodies are in a native state, not misfolded [22••]

Sec bodies are also pro-survival. When they are prevented to form, the starved cells survive less well than the control cells and they do not recover upon re-feeding. One of the reasons is that that Sec bodies also act as a protective device for COPII components and Sec16. When Sec bodies cannot form, these proteins are degraded [22••].

Mechanism: signaling through dPARP16

How do Sec bodies form? Although the Sec body proteins appear to be in a native state, they behave differently, as they no longer form COPII coats at ER exit sites. They could therefore be specifically modified upon amino-acid

Figure 3



Two distinct pathways lead to the formation of stress assemblies with different physical properties.
In growing yeast, Gcn3 (the translation initiation factor eIF-2B subunit alpha, green) is soluble. When energy is depleted, the intracellular pH drops, resulting in the formation of a higher-order assembly in a matter of minutes. A large subset of cytosolic proteins undergo the same process, leading to a solid-like cytoplasm [5••].
In growing *Drosophila* S2 cells, Sec16 (red) is bound to ER exit sites (ERES) where it binds COPII subunits (blue octagons) and regulates COPII-coated vesicle dynamics. Upon amino-acid starvation, the mono-ADP ribosylation enzyme dPARP16 is activated and modifies (black star) Sec16 on a sequence near its C-terminus (SRDC). This is necessary and sufficient to form liquid-like membrane-less Sec bodies populated by Sec16, COPII subunits and other cytoplasmic proteins (orange squares) in 2–3 hours [43]. Importantly, the formation of both assemblies is critical for cell survival during the stress period and fitness upon stress relief.

starvation, thus conferring them properties compatible with their incorporation into Sec bodies.

As ADP-ribosylation was suggested to be associated with stress granule dynamics in mammalian cells [38••,39–41], this modification has been investigated in the context of Sec body formation. This led to the identification of the mono-ADP-ribosylation (MARylation) enzyme *Drosophila* PARP16 as a key factor for Sec body formation. Importantly, dPARP16 is a survival factor for *Drosophila* cells upon amino-acid starvation [42••].

dPARP16 is the orthologue of the mammalian PARP16 that is localized at the ER [43,44] and activated by ER stress [44]. In the context of amino-acid starvation in *Drosophila*, dPARP16 MARylates Sec16 and Sec16 MARylation is both sufficient and necessary for Sec body formation (Figure 3). Interestingly, MARylation occurs on a short conserved peptide at the C-terminus of Sec16. This is reminiscent of the identification of a short sequence within proteins that tend to form Amyloid-like bodies in the nucleus of stressed cells [23••]. Assembly of these A bodies also appears to require a specific long non-coding RNA and in this regard, mono-ADP-ribose and RNA could have a similar role in mediating coalescence.

How dPARP16 is activated by amino-acid starvation remains to be elucidated. In principle, it could be activated by a drop in intracellular pH (as in starved yeast) but this drop is very rapid (at least in yeast), whereas Sec body formation takes several hours.

Taken together, these two examples illustrate that the formation of stress assemblies can be elicited by both changes in the internal conditions of cells (drop in the cytoplasmic pH) directly elicited by external fluctuations, and sophisticated signaling pathways that transmit environmental fluctuations from the outside (such as dPARP16 activation by amino-acid starvation) (Figure 3).

Higher-order assemblies are particularly abundant in stressed cells

Higher-order assemblies are frequently found in cells that are exposed to stress. What could be the function of these assemblies?

We suggest that these assemblies help cells to adapt to stress and that assembly formation has many advantages over other modalities of stress adaptation: First, assembly formation is a cooperative process. The cooperativity of many macromolecules allows switch-like responses to stress, thus enabling fast and collective decision-making. Second, assembly may be more energy efficient than regulating all these proteins through PTMs or degradation. Third, assembly formation can occur rapidly within minutes as in starved yeast, a timescale much faster than transcription or translation. This makes this an ideal mechanism for adaptation to sudden changes in the environment. Cells achieve this by promoting slight changes in the proteins that are incorporated into the assemblies. Although the time frame of assembly in amino-acid starved *Drosophila* cells appears to be much slower than in energy-deprived yeast, the same principle may apply. What is slow in *Drosophila* is presumably the drop in amino acid levels below a critical threshold. Only then signaling pathways are activated, which triggers a rapid assembly of components by phase separation. Fourth, stress assemblies are quickly reversible upon stress relief, a property compatible with the notion that proteins are assembled in a near-native state and therefore are readily re-usable. Fifth, stress assemblies protect key components from degradation, so that re-entry into the cell cycle is expedited upon stress relief, a key step in the recovery from cellular crisis. The formation of reversible stress assemblies and a change in the material properties of the cytoplasm appear to be the solution.

Different life styles shape the stress response

Why do two different organisms form reversible stress assemblies with very different properties, one that is gel-like or glass-like as in starved yeast and one that is liquid-like as for Sec bodies and stress granules in *Drosophila*? Because these organisms live in very different environments, the selection pressures shaping the evolution of their stress-adaptive assemblies may differ tremendously.

Budding yeast prefer to form solid-like assemblies

The free-living budding yeast lives in a highly fluctuating environment. This may have created strong selective pressures to evolve a proteome with rich colloidal behavior, prone to promote the formation of diverse solid-like assemblies. The ability to dissolve such solid-like assemblies is critical for survival. Cells that fail to disassemble stress assemblies cannot re-enter into the cell cycle. Thus, there may be strong evolutionary pressure to optimize not only their formation but also their dissolution.

Dissolution appears to be regulated by ATP-driven chaperones, disaggregases, and helicases [10••,23••,45,46]. For instance, budding yeast have a very active chaperone machinery, in which the disaggregase Hsp104 plays a major role [10••,46]. Yeast may share this ability with many other organisms, such as plants or protists, where the disaggregase is strongly upregulated upon stress. This may ensure recovery from a stress-induced solid-like state of their cytoplasm [47,48].

This suggests that recovering cells invest a lot of energy into dissolving stress assemblies through activating ATPases. This is in contrast to the relative energy independence of the assembly phase, which in budding yeast seems to be mostly driven by acidification (starvation) or other triggers such as changes in thermal energy (heat shock). However, the requirement for these ATPases

may differ from stress to stress. For example, starvation-induced assemblies only show a slight dependence on chaperones and may primarily depend on a rebounding cytosolic pH [10••]. In contrast, heat-adapted budding yeast strongly upregulate molecular chaperones, which may serve as disassembly factors in the recovery phase [10••,45,49••]. Similar findings have been made for other organisms such as *Drosophila* [50].

Animal cells prefer to form liquid-like assemblies

In contrast to yeast, mammalian cells rarely experience extreme changes in temperature (except for temperature fluctuations that epithelial cells experience in the mouth or nose) or energy levels, and they may have invented different assembly mechanisms to adapt to stress. A recent study provides support for this idea. Stress granules from yeast and mammalian cells were found to have very different properties: whereas yeast stress granules are solid-like, mammalian [10••] and *Drosophila* [22••] stress granules have liquid-like properties. We speculate that more stable internal conditions of animal cells have led to the development of assemblies with more liquid-like properties. These structures are easier to dissolve and may not require a disaggregation machinery. Consistent with this idea, Hsp104-like protein disaggregases have been lost in animal cells. However, other mechanisms such as phosphorylation by DYRK3 kinases seem to be required for the dissolution of stress granules in mammalian cells [35].

Nevertheless, the ability to form solid-like assemblies is not completely absent from mammalian cells. The nucleolus of mammalian cells immobilizes many growth-associated proteins under conditions of stress through the formation of solid amyloid-like assemblies (so called A bodies) [23••]. Like in yeast, formation of this stress assembly is induced through heat stress or through acidosis and recovery requires the action of chaperones. Importantly, there is a form of metabolic intracellular acidification that triggers a dormant state in many tumor cells, and this coincides with the formation of A bodies. Remarkably, genetic abrogation of A body formation leads to the loss of tumor dormancy and to more aggressive growth. Thus, it seems that some simple aspects of acidification-induced dormancy are conserved from yeast to man.

Conclusion

The formation of membrane-less compartments and stress assemblies appears to be a common and widely used method for ensuring protection of key cellular materials, promoting cell survival during stress, and their swift recovery upon stress relief (Figure 3). The two examples presented here illustrate three major differences: the differential requirement for signaling pathways in their formation; the time frame of their formation; and the different material properties. This sets the stage for future studies.

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