Spotlight

Intrinsically Disordered Proteins Adaptively Reorganize Cellular Matter During Stress

Sreenivas Chavali,1,* Alexander Gunnarsson,1 and M. Madan Babu1,*

Intrinsically disordered proteins (IDPs) can protect cells from diverse stresses by forming higher order assemblies such as reversible aggregates or granules. Recently, Boothby et al. show that IDPs protect tardigrades against desiccation by forming a glass-like amorphous matrix, highlighting that material properties of disordered proteins can confer adaptation during stress.

The specific function(s) of a protein is affected by its structure, which is determined by its sequence and the appropriate conditions. Organisms are constantly subjected to environmental changes, which include diverse stress conditions such as fluctuations in temperature, levels of oxygen, water and nutrients. Drastic changes in conditions lead to protein unfolding, denaturation, aggregation and degradation and affect cellular fitness. How do organisms adapt to the diverse stress conditions? One mechanism involves expressing stress response proteins. For instance, heat shock proteins (HSPs) expressed during temperature stress inhibit non-physiological protein aggregation and help maintain the functional structures of proteins [1]. Although there is a broad consensus about the expression of certain stress response proteins, what constitutes the stress response proteome and the mechanisms of protection have largely remained elusive.

To address these questions, Boothby et al. study how tardigrades adapt to desiccation [2]. Tardigrades, commonly known as water bears, are microscopic, cryptobiotic invertebrates that survive extreme conditions such as complete desiccation for up to a decade. The necessity of slow drying (preconditioning) to survive subsequent rapid drying highlights that sufficient time is required to produce molecular protectants against desiccation. Differential gene-expression analysis in the tardigrade Hypsibius dujardini identified upregulation of several transcripts encoding cytosolic abundant heat soluble (CAHS) and secreted abundant heat soluble (SAHS) proteins in dry versus hydrated conditions.

By integrating data from computational predictions, Nuclear Magnetic Resonance (NMR) spectroscopy, published data on heat solubility, and circular dichroism, the authors identified that many CAHS and SAHS proteins are likely to be intrinsically disordered proteins (IDPs), in that they lack persistent tertiary and/or secondary structure. These proteins are referred as tardigrade-specific IDPs (TDPs). Constitutive or differential expression of TDPs during drying among other tardigrades such as Paramacrobiotus richtersi and Milnesium tardigradum suggest that the TDP-mediated protection might be evolutionarily conserved. By disrupting the expression of TDPs using RNA interference, Boothby et al. established that TDPs are indeed required for desiccation tolerance. Strikingly, several TDPs were individually sufficient to increase desiccation tolerance when expressed in heterologous systems such as yeast and bacteria. Remarkably, purified TDPs could preserve the enzymatic activity of lactate dehydrogenase upon desiccation in vitro, showing that TDPs are sufficient to prevent protein denaturation and loss of function.

How do TDPs protect biological material from desiccation? Differential scanning calorimetry investigations revealed that CAHS proteins vitrify when dried and form a glass-like matrix, both in vitro and when expressed in yeast. Vitrification is thought to trap proteins within the pores of the amorphous matrix, which protects them from denaturation. Upon rehydration, the physiological state of the cell seems to be restored, with the dissolution of this reversible glassy matrix. Similar mechanisms are also observed in plants, where the LEA family of disordered proteins vitrifies alongside sugars to protect seeds and roots from drought [3]. The fact that both plants and tardigrades have convergently evolved a similar process to protect against desiccation suggests that certain disordered proteins might confer specific material properties for stress adaptation.

Accumulating evidence has established that intrinsically disordered proteins can organize biological matter in the cell as a response to diverse stresses. For instance, stress granules are membrane-less organelles that can sequester and protect both RNAs and proteins from stress-induced damage [4,5] (Figure 1A). The sequestration of signaling and regulatory proteins can alter signaling pathways during stress as seen for mammalian/mechanistic Target of Rapamycin Complex 1 (mTORC1) [6]. Similarly, regulated, reversible aggregation during heat stress involves nucleic acid-protein and protein-protein interactions [7] (Figure 1B). This process involves several proteins that form stress granules, including the Poly(A) binding protein [5]. The finding that an all protein-mediated vitrification can act as a protective mechanism adds another critical insight to this emerging new field of phase transition that is mediated by disordered proteins in response to stress (Figure 1C). Thus, a major challenge is to not only discover how many such assemblies exist in cells but also to resolve these assemblies in terms of their composition, molecular driving forces, as well as their dynamics [8–10].

What properties of IDPs facilitate assembly formation and protection against
An emerging view is that low complexity disordered protein sequences with amino acid compositional bias facilitates phase separation. Consistent with this, 10 of the 12 TDPs contain compositionally biased low complexity regions. Furthermore, specific combinations of charged and hydrophobic residues (that can form cation-pi interactions), the occurrence of multiple short linear motifs, as well as post-translational modifications can all facilitate and regulate formation of reversible assemblies. 

The unusual properties of IDPs [12], both in terms of their conformational flexibility and evolvability, might have allowed them to become molecular protectants during stress by organizing and protecting biological matter. However, the challenge now is to fully understand the extent of these functions and their limits. Importantly, unregulated phase separation of IDPs has been implicated in several diseases. For instance, toxic vitrified states can lead to neurodegeneration. Thus a key question is what attributes of IDPs distinguish the toxic irreversible assemblies from the protective reversible vitrification as seen in tardigrades. The protective mechanisms described in tardigrades and other organisms allude to many promising applications such as those in engineering drought resistant crops and preservation of functional protein therapeutics in the dry state. This study highlights how gaining a molecular understanding of basic processes such as the stress response in an unusual model organism can lead to exciting discoveries with broad implications in biotechnology and potentially in biomedicine.

**Figure 1.** Reversible Reorganization of Cellular Matter by IDPs during Stress. (A) Under non-stress conditions, the yeast IDP poly(A) binding protein (Pab1) is bound to RNA and represses translation. Pab1 phase separates and forms hydrogels in response to thermal and pH stresses. Electrostatic and hydrophobic interactions within disordered segments promote phase separation. During recovery, Pab1 is resolubilized and rebinds RNAs to repress translation. Under severe stress, Pab1 along with other quinary assemblies localize to stress granules [5]. (B) Upon heat shock, IDPs such as Gbp2, Fpr3 and Gus1 are involved in the formation of reversible aggregates (heat shock granules) in yeast. About 170 endogenous proteins have been identified to aggregate in distinct subcellular compartments during heat stress. Disaggregation during recovery at non-shock temperatures leads to near-complete reversion of the aggregate into a stable soluble pool of active proteins. Thus, heat-induced aggregation of proteins is an adaptive process that confers stress tolerance [7]. (C) In Tardigrades, IDPs vitrify and form amorphous matrix to protect cellular matter from drying during desiccation [2].
References