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SPOTLIGHT

One domain fits all: Using disordered regions to sequester misfolded proteins

Edgar E. Boczek and Simon Alberti

Small heat shock proteins (sHsps) are adenosine triphosphate–independent chaperones that protect cells from misfolded proteins. In this issue, Grousl et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201708116) show that the yeast sHsp Hsp42 uses a prion-like intrinsically disordered domain to bind and sequester misfolded proteins in protein deposition sites.

Cells exposed to stress respond in various ways to maintain protein homeostasis, or proteostasis. One central mechanism is known as spatial protein quality control. This involves the sequestration of misfolded proteins in deposition sites. Budding yeast, a frequently used model organism for the study of spatial protein quality control, has two deposits for misfolded proteins that are referred to as intranuclear quality control compartments (INQs) and peripheral cytosolic aggregates (CytoQs). The formation of INQs and CytoQs is facilitated by two dedicated protein-sorting factors called Btn2 and Hsp42, respectively (Specht et al., 2011; Malinovska et al., 2012).

Hsp42 is a highly promiscuous ATP-independent chaperone that can interact with 30% of the yeast proteome (Haslbeck et al., 2004). It belongs to the group of small heat shock proteins (sHsps), which often form a first line of defense during stress, keeping misfolded proteins in a native-like and refolding-competent state (Haslbeck and Vierling, 2015). Upon release from sHsps, cellular disaggregation machineries can refold the substrate proteins in an ATP-dependent manner (Mogk et al., 2018). Similar to other sHsps, Hsp42 can act as an aggregation-preventing chaperone when present in excess. However, Hsp42 is unique in its ability to act as an aggregation-promoting factor (an aggregase) in vitro at substoichiometric sHsp/substrate ratios (Ungelenk et al., 2016). How Hsp42 mediates the formation of CytoQ aggregates in the cell has so far remained elusive. In their study, Grousl et al. discovered a coordinated role for intrinsically disordered regions of Hsp42 in the regulation of protein aggregation.

Like all members of the sHsp family, Hsp42 is comprised of a central folded α-crystallin domain (ACD) and disordered N and C termini (Haslbeck and Vierling, 2015). The disordered N-terminal domain of Hsp42 is unusually long and known to be essential for CytoQ formation (Specht et al., 2011). The N terminus of Hsp42 is also remarkable because of its prion-like amino acid composition (Alberti et al., 2009). Prion-like domains (PrLDs) are low-complexity sequences enriched for polar amino acids such as glutamine, asparagine, and tyrosine. PrLDs are often found in aggregation-prone proteins and have been implicated in the formation of membraneless compartments (Franzmann et al., 2018). Like the PrLDs in many other prion-like proteins, the PrLD of Hsp42 is followed by a disordered charged region called the intrinsically disordered domain (IDD).

In their study, Grousl et al. (2018) used an intrinsically unstable VHL-mCherry protein as an in vivo misfolding-prone model substrate and a marker for protein deposits. Upon proteotoxic stress, VHL-mCherry formed one or two CytoQs in yeast cells. These CytoQs assembled from many smaller aggregates, which merged into one or two deposits over time. Unexpectedly, Grousl et al. (2018) found that in the presence of cycloheximide, a potent blocker of protein synthesis, CytoQs no longer formed. This suggests that the majority of CytoQ substrates are newly synthesized proteins. This surprising result raises important questions. For example, how does Hsp42 specifically target newly synthesized proteins, and is Hsp42 directly linked to ribosome quality control systems?

Grousl et al. (2018) then went on to study the role of the disordered N-terminal domain of Hsp42 in detail. Because the long N-terminal region of Hsp42 can be divided into a PrLD and an IDD region, they created two deletion variants lacking either the PrLD (ΔPrLD) or the IDD (ΔIDD; Fig. 1). When they tested ΔPrLD in cells, CytoQ formation could no longer be observed. The same result was found for a mutant in which all tyrosines in the PrLD had been exchanged for serines, thus suggesting a key role for aromatic side chains in CytoQ formation.

In contrast, small CytoQ aggregates still formed in the absence of the IDD even in nonstressed cells. However, the subsequent fusion of CytoQs into larger deposits was impaired as well as their disaggregation after stress release. This suggests that the IDD region of Hsp42 modulates the material properties

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Correspondence to Simon Alberti: alberti@mpi-cbg.de .

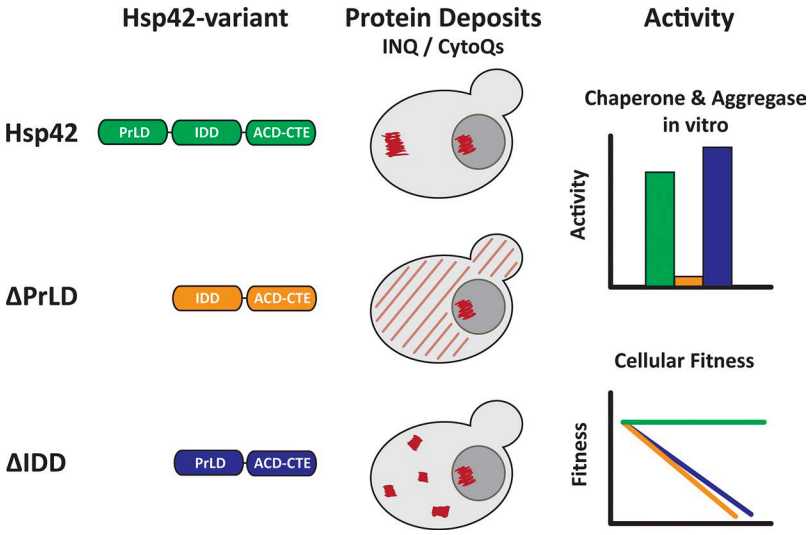

Figure 1. **Effects of domain deletions in Hsp42.** WT Hsp42 (green) efficiently sequesters misfolded proteins in one or two CytoQ sites in the cytoplasm. In vitro, it is active as a chaperone and as an aggregase and sustains cellular fitness during stress. The ΔPrLD mutant (orange) lacks the substrate-binding site and thus fails to act as a chaperone or an aggregase in vitro and in vivo. As a consequence, the cells show decreased fitness. Deletion of the IDD in the ΔIDD mutant (blue) results in increased chaperone and aggregase activity and increased CytoQ formation in vivo. However, cellular fitness is similarly impaired in this mutant, presumably because the formed aggregates have different material properties. ACD-CTE, ACD and C-terminal extension domain.

of the CytoQ aggregates. Importantly, deletion of both the PrLD or the IDD had a strong impact on cellular survival under stress conditions.

To identify the molecular mechanisms behind these observations, Grousl et al. (2018) exploited an extensive arsenal of in vitro methods. Using a chaperone activity assay based on the model substrate malate dehydrogenase (MDH), they found that the ΔPrLD mutant had lost its ability to act as a chaperone. In contrast, the ΔIDD mutant showed a similar chaperone activity as the WT protein in a conventional turbidity assay and even higher activity in a more sensitive fluorescence resonance energy transfer assay. Using hydrogen/deuterium exchange coupled with mass spectrometry, Grousl et al. (2018) further found that Hsp42 stabilizes a native-like state of MDH, with the ΔIDD mutant showing even stronger stabilization. Together with the finding that ΔIDD–MDH complexes were smaller, this suggests that the chaperone acts on early misfolding intermediates and that the stoichiometry of the chaperone–substrate complex is altered in the IDD-lacking mutant. Notably, the increased chaperone activity was not related to higher monomer exchange rates as these were similar to WT Hsp42.

In another set of experiments, Grousl et al. (2018) used cell lysate pulldown experiments and chemical cross-linking coupled with mass spectrometry to study the interaction of Hsp42 with substrate proteins. They found, remarkably, that the PrLD directly interacts with misfolding-prone proteins and that the ΔPrLD mutant suffered from a complete lack of chaperone activity. Surprisingly, deletion of the IDD increased the binding of Hsp42 to misfolded proteins. It will be interesting to extend the mapping of interactions to the central ACD because this region was shown to interact with substrates in other sHsps (Haslbeck and Vierling, 2015).

These findings raise an important question: how does the IDD modulate substrate interactions with the PrLD? A potential explanation comes from 8-anilinonaphthalene-1-sulfonic acid binding assays showing that the IDD may shield hydrophobic patches in the PrLD that may be involved in substrate binding. Grousl et al. (2018) provide further evidence that Hsp42 molecules can also self-interact via PrLD–PrLD interactions, and the size of the resulting oligomers was modulated by the IDD. Consistent with this, further in vitro experiments revealed an increased ability of the ΔIDD mutant to sequester misfolded proteins as compared with the WT, whereas the ΔPrLD mutant completely lacked this aggregase ability. This suggests that the chaperone activity of Hsp42 and its aggregase function are tightly linked.

The picture emerging from the study by Grousl et al. (2018) is that Hsp42 binds to substrates via its PrLD and that this facilitates CytoQ formation. Without the IDD, the formed aggregates are in a state that is incompatible with CytoQ maturation and rapid dissolution via Hsp104. The adjacent IDD in Hsp42 also dampens the substrate-binding affinity of the PrLD, leading to more fine-tuned chaperone and aggregase activity. As both the ΔPrLD and the ΔIDD mutants show a similar fitness defect, the process of CytoQ formation and maturation seems equally important as the chaperone and aggregase activities of Hsp42. In fact, the findings suggest that a balance between the activities of the PrLD and IDD is crucial for the function of Hsp42 in vivo (Fig. 1).

This remarkable and very insightful study opens up several novel research avenues for the future. Why are many CytoQ aggregates more harmful to the cell than a few large ones? Are these effects related to the volume-to-surface ratio of the particles? Does the IDD tune the material properties of CytoQ aggregates, thus allowing the merging of small aggregates into one or two large CytoQs? Is the removal of misfolded proteins from CytoQs through Hsp104 impaired in the absence of the IDD? A careful investigation of the material properties of CytoQs and their interactions with other cellular components such as Hsp40, Hsp70, and Hsp104 may give important clues.

The findings presented in this paper are in many ways reminiscent of a recent study showing that the translation termination factor Sup35 forms stress-protective compartments by phase separation (Franzmann et al., 2018). The formed compartments have gel-like properties, and they protect the essential termination factor from damage. Like Hsp42, Sup35 has an acidic region adjacent to its PrLD, and this region modulates the assembly of the PrLD in response to changes in pH. Thus, one possibility is that the IDD region of Hsp42 is also a sensor

domain that responds to changes in the environment. Indeed, Hsp42 also forms CytoQs in cells that have been exposed to glucose starvation, and this is one of the conditions under which the cytosolic pH changes significantly (Munder et al., 2016). Another possibility is that the IDD is regulated by posttranslational modifications.

Important questions remain to be investigated: What causes the Hsp42–substrate complex to aggregate? What is the fraction of substrate–substrate, substrate–chaperone, and chaperone–chaperone interactions in these aggregates? How do the structure and conformational dynamics of Hsp42 change upon substrate binding or deletion of the IDD? How does the IDD modify chaperone–chaperone and chaperone–substrate interactions? Do the oligomers that form in the absence of the IDD domain have an altered number of substrate-binding sites, or is the number of sites for chaperone–chaperone interactions different?

Finally, it will be exciting to test whether these findings extend to other sHsps. All sHsps share the conserved structural attributes of an ACD and a significant portion of disordered regions at the N and C termini. Many sHsps are also characteristically enriched for arginines, prolines, and hydrophobic residues in their N termini. Dissecting the role of these disordered regions and amino acid motifs for chaperone function seems especially important in the light of the fact that sHsps are involved in many neurodegenerative diseases.

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