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When proteostasis goes bad: protein aggregation in the cell

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ABSTRACT

Protein aggregation is a hallmark of the major neurodegenerative diseases including Alzheimer's, Parkinson's, Huntington's and Motor Neuron and is a symptom of a breakdown in the management of proteome foldedness. Indeed, it is remarkable that under normal conditions cells can keep their proteome in a highly crowded and confined space without uncontrollable aggregation. Proteins pose a particular challenge relative to other classes of biomolecules because upon synthesis they must typically follow a complex folding pathway to reach their functional conformation (native state). Non-native conformations, including the unfolded nascent chain, are highly prone to aberrant interactions, leading to aggregation. Here we review recent advances in knowledge of proteostasis, approaches to monitor proteostasis and the impact that protein aggregation has on biology. We also include discussion of the outstanding challenges.

Nuts and bolts of proteostasis

As newly minted proteins emerge from the ribosome they face a series of challenges to correctly fold and be transported to their subcellular destination. Nascent unfolded proteins are inherently "sticky" and emerge into a very densely crowded molecular environment, which heightens the prospects of folding being side-tracked by competing aggregation processes. The maintenance of proteome foldedness, function and solubility (proteostasis) is governed by an extensive collection

of proteins that form a proteostasis network (PN). The PN includes quality control machinery that shield proteins from non-native interactions, act as folding checkpoints, and govern protein synthesis, turnover and trafficking [1, 2] (shown conceptually in Figure 1).

During protein synthesis, hydrophobic regions of nascent polypeptide chains are protected from non-specific interactions by ribosome-associated chaperones. This includes the nascent chain associated complex, Hsp70 chaperones and Hsp40 co-chaperones. Indeed Hsp70-family chaperones form key hubs of the PN and can be tuned for functional specificity within the PN by linking to diverse adaptor proteins and hsp40 cofactors as well as directing protein targets for degradation [3-7]. Hsp70 family proteins are also important in preventing misfolding of nascent chains during translation and ensuring optimum translation rates [8, 9]. Quality control machinery also survey mRNA and nascent proteins for faults. This includes the recently described Ribosome Quality Control Complex (RQC) in yeast, which senses and clears stalled nascent protein-ribosome complexes [10] and non-sense mediated decay or no-go decay that degrade stalled proteins on faulty mRNA sequences [11, 12].

The classic theory of protein folding thermodynamics has been established from studies of soluble globular proteins that assume the process is largely reversible [13, 14]. These proteins adopt an equilibrium between the native fold and a myriad of unfolded and partially folded states [15]. They have evolved to establish the native state as the most energetically favoured conformation under physiological conditions, which can be defined by a negative Gibbs free energy of this equilibrium [15, 16]. However, more complex proteins, notably membrane proteins, have far more elaborate and distinct folding mechanisms requiring a different theoretical framework that is still not well established [17]. What is clear however is that they rely absolutely on the PN to fold in incremental stepwise manners and that this is typically not a reversible process. In bacterial systems, folding of membrane proteins can be mechanistically modeled to require chaperones to drive efficient folding into the membrane [18]. In eukaryotes, the classic pipeline of complex protein folding includes production of proteins in the endoplasmic reticulum, regulation of foldedness by the Calnexin cycle, which involves glycosylation modifications, further post-translational modification in the Golgi before delivery to the target location in the cell [19]. Collectively, more than two thirds of the proteome are considered “complex” and hence significant energy must be invested by the cell via the PN to manage the folding of these proteins [20]. This is reflected by the large number of

proteins (318 in humans at the time of writing) in the Gene Ontology Consortium term of “Protein Folding; GO:0006457”, which includes many proteins of the PN.

Failure of proteostasis and protein aggregation

Unfolded states are permissive to entering misfolding pathways that result in aggregation, which provides a rival low energy state to the native fold [21]. For this reason, aggregation is often observed as symptomatic of problems in maintaining proteostasis [22, 23]. For example, proteins accumulate into microscopically visible aggregate structures inside neurons, known as inclusions, as a hallmark of neurodegenerative diseases including Alzheimer’s disease, amyotrophic lateral sclerosis and Huntington’s disease [24]. Proteins may also naturally aggregate during the normal ageing process, with the PN remodeling to accommodate the changes — a process that may be maladapted in neurodegenerative disease [25-27]. Mutations can also change the folding stability of individual proteins, and may pose as modifiers of baseline proteostasis capacity that influence disease risk [28, 29].

One of the great challenges of current research efforts is in understanding how protein aggregation relates to toxicity. Many studies support a hypothesis for soluble protein oligomers, as precursors to a larger amyloid state, as directly proteotoxic and that this seems to be independent of the protein sequence [30, 31]. Larger aggregates seem to be less toxic, potentially due to a lower concentration of reactive “ends” of fibrils [30, 32-34]. However, the mechanisms for toxicity, and the relevance to disease remain to be robustly validated [35]. Possible routes of toxicity include physical disruption of membranes or other cellular structures, or interference with synaptic structure and plasticity [36, 37]. Of note is a recent study showing that the nuclear pore is fundamentally damaged in cells that display extensive cytoplasmic aggregates of unrelated aggregation-prone proteins [38]. Components of the nuclear import and/or export machinery coaggregate with disease-associated mutant proteins including polyglutamine-containing huntingtin protein, mutant TDP-43 and the C9ORF72-associated polydipeptides [38-40]. Indeed, the progressive loss of proteostasis may account for the observation that nuclear transport becomes increasingly “leaky” with ageing [41].

One of the more interesting recent hypotheses for proteotoxicity of protein aggregates arises from the observation that markers of stress granule abnormalities appear in pathology (notably Motor Neuron Disease) [42]. Indeed several RNA granule proteins, most notably TDP-43, FUS and HNRNP family proteins cause Motor Neuron Disease when mutated [43-45]. Stress granules, and

related structure P-bodies, are condensed foci of mRNA and ribonucleoproteins that form under translational stresses [42]. They act as sites for temporal translational repression and mRNA-ribonucleoproteins quality control processing. Recent studies have suggested many RNA granule proteins contain predicted prion-like domains that mediate liquid:liquid protein phase separation and/or "functional" amyloid scaffolding [46-48]. The presence of "functional amyloid" indicates a necessity for these structures to be rigorously tamed to avoid pathological aggregation into amyloid fibrils. Indeed, mutations or conditions that tip the balance to pathological aggregation has been proposed as a basis for these neurodegenerative diseases [49].

Sequestration mechanisms to protein misfolding stress

In line with the theory that soluble oligomers may be toxic, there is evidence that sequestration of misfolded proteins into very large aggregates can mitigate toxicity. The most compelling evidence for this model comes from investigation of how the exon 1 fragment of mutant Huntingtin, which accumulates as visible aggregates (inclusions) in Huntington disease [50-53], aggregates in cell culture models. Cells that formed inclusions had greater rates of survival than cells that did not [33]. The mechanisms for this effect with Huntingtin exon 1 remain to be determined, however, similar "active" mechanisms of inclusion building have been proposed with other misfolded proteins. The original model was that of the "aggresome", which described a dynein-mediated retrograde transport mechanism of misfolded ΔF508 Cystic Fibrosis Transmembrane conductance Regulator protein into the microtubule organizing centre [32, 54]. The aggresome model, however, is problematic in that it does not appropriately define the diversity of processes that sequester aggregating proteins into a centralized location. More recent studies have partly addressed this deficiency by unearthing two distinct aggresome-like compartments for aggregating proteins. One is the juxtanuclear quality control (JUNQ), which comprises reversibly-aggregated proteins, and the other is the insoluble protein deposit (IPOD), which comprises irreversibly-aggregated proteins [34]. Different disease-associated aggregating proteins seem to preferentially partition into either JUNQ or IPOD exclusively, and that these compartments may correlate with different mechanisms of aggregation[55]. Huntingtin exon 1 accumulates in IPOD-like structures. By contrast, polyalanine, which aggregates via soluble α-helical clusters, and SOD1 mutants, which also cluster into soluble oligomers prior to aggregation, accumulate in JUNQ-like structures [55, 56]. FUS and TDP-43 partitioned into both structures as well as another unidentified structure [57]. These data

indicate that aggregation into foci arises via diverse processes, which are in part consistent with the JUNQ and IPOD models, but which also seem to involve a further uncharacterized layer of complexity. Hence, critical questions remain as to what factors drive proteins to different compartments, how the elements of different models fit together, whether there are further inclusion types that remain to be discovered, and how the additional inclusion types relate to the JUNQ and IPOD structures.

An omics view of proteostasis

Omics technologies, along with new computational approaches have begun to transform our understanding of what happens to the proteome under proteostasis stress. For example, these approaches have led to insight in how hundreds of proteins aggregate under stress in different organisms and cell culture conditions, [25, 58-61]. One common thread in the data is that distinct stress types tend to induce the same proteins to aggregate by reducing their aggregation threshold, which indicates the presence of metastable sub-proteome [61-63]. This includes many regulators of proteostasis, such as chaperones, and proteasome subunits [25]. Another study investigated the properties of proteins that aggregated during ageing in *C. elegans* [60]. The proteins that contributed most extensively to aggregation had relatively low intrinsic aggregation propensity but were so highly abundant in the cell that they were supersaturated with respect to their solubility. A supersaturation score, which combines experimental and bioinformatics data, has been used to explain which proteins coaggregate with amyloid plaques, neurofibrillary tangles, Lewy bodies and artificial β-proteins, as well as proteins that aggregate in *C. elegans* during ageing [64].

In spite of the many proteins abnormally aggregating under stress, there is evidence also for an adaptive aggregation response in parallel. For example, under heat stress, many proteins form transiently aggregated complexes that remain functionally competent, and that are not degraded upon recovery [58, 59]. Such structures were suggested to aid in the management of the stressed proteome [59].

Quantification of proteostasis

A major area of development in the study of proteostasis involves building quantitative tools to mechanistically probe the PN. One approach to do this relies on metastable reporter proteins that engage with the PN and provide a read out of how well it suppresses aggregation as an indicator of proteostasis efficacy. This includes temperature-sensitive endogenous proteins, which have been

used to identify novel regulators of the PN in *C. elegans* [65] and to probe the collapse of proteostasis when disease-associated proteins are expressed [31]. More advanced strategies involved designer ectopic reporters including destabilised mutants of firefly luciferase, which is a known chaperone substrate that requires Hsp70 and Hsp90 to fold [66], and the *de novo* designed enzyme retroaldolase[67] as a sensor of proteome stress [68]. These reporters offer the advantage of not interfering with normal biological pathways and have been used in these above cited studies to probe changes in proteostasis induced by drugs, disease proteins, and ageing.

Other approaches have used small molecules to probe the foldedness of target proteins. In one example, reactive ligands to two proteins transthyretin and retroaldolase were used to measure the extent of functionally competent protein in *E. coli* lysate out of the total pool of each protein (which included unfolded and aggregated states) [68, 69]. The work revealed a significant fraction of these proteins to be unfolded, which was attributable to a holdase effect of proteostasis machinery. Indeed manipulation of proteostasis levels with DnaKJE chaperone and co-chaperone overexpression increased the holdase effect whereas overexpression of the GroEL/ES chaperonin system increased foldedness. These outcomes are in accordance with the known mechanisms of these chaperone systems and indicates the utility of this strategy to monitor proteostasis.

Another strategy for insight to proteostasis has been the systems approach of computationally modelling the entire PN. This strategy offers mechanistic insight to the connections between different modules of the PN in a way that makes it possible to make sense of experimental data and make predictions that informatively guide experiments. FoldEco provides a comprehensive computational model of the PN in *Escherichia coli* [70]. This platform provides the capacity to model, and make prediction of, the kinetics of chaperone-protein interactions as well as synthesis, aggregation and degradation in a fully integrated system. It can also be "fitted" to experimental data for mechanistic understanding of proteostasis [71]. However, transferring this approach to eukaryotic cells remains an ongoing challenge because of their vastly more complicated proteostasis network in terms of the number of proteins involved and greater levels of compartmentalization of the networks in organelles such as the ER.

The path ahead in this research

The true potential of proteostasis measurement will be realized when computational models can be integrated with advanced tools for measuring proteostasis activity. Further work, which our lab is

actively engaged in, will be required to develop extremely well characterized sensors that are able to probe the diverse arms of the proteostasis network without disrupting it, and yield quantitative information that can feed into network models that enable information-rich analysis.

Small molecules that bind specifically to the folded conformation are promising not only as reporters of the folded conformation, but also as pharmacological chaperones (PC) that stabilise the native state and have potential as therapeutics for protein misfolding diseases. PC can stabilise the native conformation either thermodynamically or kinetically [72]. Thermodynamic stabilisation shifts the equilibrium toward the native state and thereby increases the concentration of functional protein. This approach has potential to treat loss-of-function diseases and has shown good promise in treatment of a variety of lysosomal storage disorders [73]. Kinetic stabilisation of proteins reduces unfolding, and thereby minimises off-pathway misfolding to toxic species that drive gain-of-toxic-function protein misfolding diseases. Other similar approaches are being explored for treatment of other diseases. For example defects in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel from the Δ508Y mutation lead to a loss in folding yield. Drugs that aid folding or reduce clearance of misfolded or incompletely-folded forms by the PN are being developed for clinical use [74, 75]. Clinical trial results of a pharmacological chaperone therapy for familial amyloid polyneuropathy (caused by gain-of-toxic function of transthyretin mutants) that stabilizes the folded state tetrameric state of transthyretin show delay of neurological progression [76].

In conclusion, research into mechanisms of proteostasis and relationship to protein aggregation in disease has progressed substantially in the last few years. We see several key questions as immediate challenges to be addressed. An important question is how generalizable are the mechanisms of toxicity caused by aggregation of different proteins? Are the mechanisms of proteotoxicity non-specific? And are there certain machinery hotspots, such as the nuclear pore, that need to be “hit” to trigger disease? Another gap is defining precisely which proteins aggregate in the proteome under proteostasis collapse, and probing whether this explains the changes seen in disease. Given that TDP-43 mislocalization is observed as marker of all forms of sporadic ALS [77], is TDP-43 simply a bellwether for a broader subproteome highly prone to aggregation under stress? We see a need to build new tools and approaches that can get at such problems including

more quantitative measures of proteostasis as important steps forward in understanding this biology.

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Figure 1: Proteostasis governs the life of a protein from synthesis to degradation. Shown is a simplified diagram of key steps in the life of a protein and how this is guided by the proteostasis network under normal and stressed conditions (green). Management of protein folding is normally regulated by a network of hundreds of proteins in humans. When proteostasis fails, proteins can misassemble into aggregates. The failure of proteostasis correlates with a gain of proteotoxicity; some of the postulated mechanisms covered in this review are indicated (red)**.**