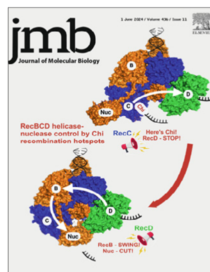


Interplay of Proteostasis Capacity and Protein Aggregation: Implications for Cellular Function and Disease

Mark S. Hipp [1,2,3,*] **and F. Ulrich Hartl** [4,5,6,*]

*1 - **Department of Biomedical Sciences,** University Medical Center Groningen, University of Groningen, Antonius Deusinglaan, 1, 9713 AV Groningen, the Netherlands*

*2 - **Research School of Behavioural and Cognitive Neurosciences,** University of Groningen, Groningen, the Netherlands*

*3 - **School of Medicine and Health Sciences,** Carl von Ossietzky University Oldenburg, Oldenburg, Germany*

*4 - **Department of Cellular Biochemistry,** Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany*

*5 - **Munich Cluster for Systems Neurology (SyNergy),** Munich, Germany*

*6 - **Aligning Science Across Parkinson's (ASAP) Collaborative Research Network,** Chevy Chase, MD, USA*

Correspondence to Mark S. Hipp and F. Ulrich Hartl: *m.s.hipp@umcg.nl (M.S. Hipp), uhartl@biochem.mpg.de (F.U. Hartl)*

https://doi.org/10.1016/j.jmb.2024.168615

Edited by J. Buchner

Abstract

Eukaryotic cells are equipped with an intricate proteostasis network (PN), comprising nearly 3,000 components dedicated to preserving proteome integrity and sustaining protein homeostasis. This protective system is particularly important under conditions of external and intrinsic cell stress, where inherently dynamic proteins may unfold and lose functionality. A decline in proteostasis capacity is associated with the aging process, resulting in a reduced folding efficiency of newly synthesized proteins and a deficit in the cellular capacity to degrade misfolded proteins. A critical consequence of PN insufficiency is the accumulation of cytotoxic protein aggregates that underlie various age-related neurodegenerative conditions and other pathologies. By interfering with specific proteostasis components, toxic aggregates place an excessive burden on the PN's ability to maintain proteome integrity. This initiates a feed-forward loop, wherein the generation of misfolded and aggregated proteins ultimately leads to proteostasis collapse and cellular demise.

© 2024 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Introduction

Proteins are responsible for almost all cellular functions. To sustain protein homeostasis, or proteostasis,[1] mammalian cells must maintain functional protein levels and ensure the timely folding, assembly, and conformational maintenance of over 10,000 distinct proteins, safeguarding their functionality in the face of various environmental and metabolic challenges. This difficult task is further complicated by the limited thermodynamic stability of proteins, rendering them prone to misfolding at physiological temperatures. Moreover, precise regulation of cellular localization and concentration is essential for many proteins. Protein levels are adjusted not only by control of protein synthesis,[2] but also by regulated degradation.[3–5] While for some proteins abundance can vary substantially without detrimental effects,[6] for others altering functional levels is critical in driving cellular processes like mitosis[7] and in regulating responses to environmental stress.[8,9]

Maintenance of proteostasis therefore involves the complex interplay of molecular chaperones, their regulators, and the proteolytic degradation machinery, forming an intricately coordinated

0022-2836/© 2024 The Author(s). Published by Elsevier Ltd.This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Please cite tts article as: M.S. Hipp and F.U. Hartl, Interplay of Proteostasis Capacity and Protein Aggregation: Implications for Cellular Function and DiseaseJournal of Molecular Biology, https://doi.org/10.1016/j.jmb.2024.168615

Proteostasis Network (PN), comprising ~2,900 different proteins in mammalian cells [https://www.proteostasisconsortium.com/pn-annotation/]. While the organizational principles of this network remain a subject of ongoing exploration, it is evident that the PN has evolved to maintain proteome integrity by controlling the levels of functional proteins (Figure 1A) and preventing the accumulation of aberrant protein conformations, notably the formation of aggregates (Figure 1B), which are associated with a spectrum of pathologies, from Alzheimer's disease (AD) to type 2 diabetes.[10–14]

Defining protein aggregates as any assembly of two or more protein molecules in a non-native conformation, it becomes apparent that there can be multiple aggregate forms, varying in structure and ranging from amorphous clusters to highly ordered amyloid fibrils with cross-beta topology, with the latter often being deposited in intracellular inclusions or extracellular plaques. Although morphologically similar at the light microscopic level, especially in fixed tissue samples, aggregate deposits differ from biomolecular condensates, which are liquid-like accumulations of functional proteins. Notably, condensate formation may precede and facilitate pathologic aggregation (see Box: Liquid-Liquid Phase Separation and Protein Aggregation).

The propensity of a given protein to aggregate primarily hinges on the physicochemical properties of its amino acid sequence, the stability of its native conformation, and its cellular concentration.[15,16] Within the densely packed cellular milieu, where proteins occupy between 15% and 35% of cell volume,[17] macromolecular crowding exacerbates the likelihood of non-native protein molecules aggregating compared to dilute solutions.[18] Additionally, more than 30% of eukaryotic proteins (>40% in humans) are predicted to contain structurally disordered regions more than 30 amino acids in length,[19] rendering them metastable and potentially prone to aggregation. Several neurodegenerative disease proteins, such as α-Synuclein and the microtubule-associated protein Tau belong to this class of proteins.[20] Aggregate deposition can serve as a general indicator of proteostasis disruption. However, the formation of aggregates not only reflects inadequate proteostasis capacity, but the aggregates, once formed, may further enhance PN imbalance by titrating the available chaperone and degradation machineries. This sets in motion a self-propagating cycle, ultimately culminating in proteostasis failure and cell death (Figure 2).

The molecular mechanisms of specific elements of the PN and the structural basis of aggregate formation have been extensively reviewed.[21–29] In this article we explore the relationship between protein aggregation and the functional integrity of the PN, focusing on recent advances. We propose a model in which the age-dependent decline in the ability of cells to sustain proteostasis and activate cellular stress responses gives rise to the formation of aggregates. These then further impair PN capacity, accelerating proteostasis decline and facilitating the manifestation of a spectrum of age-related diseases. Seeking ways to pharmacologically correct the PN imbalance underlying these conditions offers a promising avenue for the development of innovative therapeutic strategies.

Box: Liquid-Liquid Phase Separation and Protein Aggregation

A special case of disease-relevant protein aggregation involves liquid–liquid phase separation (LLPS) of certain proteins containing low-complexity domains, often with prion-like properties.[208–210] LLPS is characterized by the spontaneous demixing of molecules from a homogeneous solution. This process generates two coexisting liquid phases: a condensed phase (a membrane-free droplet-like compartment) and a depleted phase (the bulk solution). The resulting interface at the boundary of the condensed droplet selectively governs the passage of some molecules, while excluding others. These droplets can be described as membraneless compartments[211] that can compartmentalize and enhance biochemical reactions, or sequester molecules that are temporarily not needed.[113,212–214]

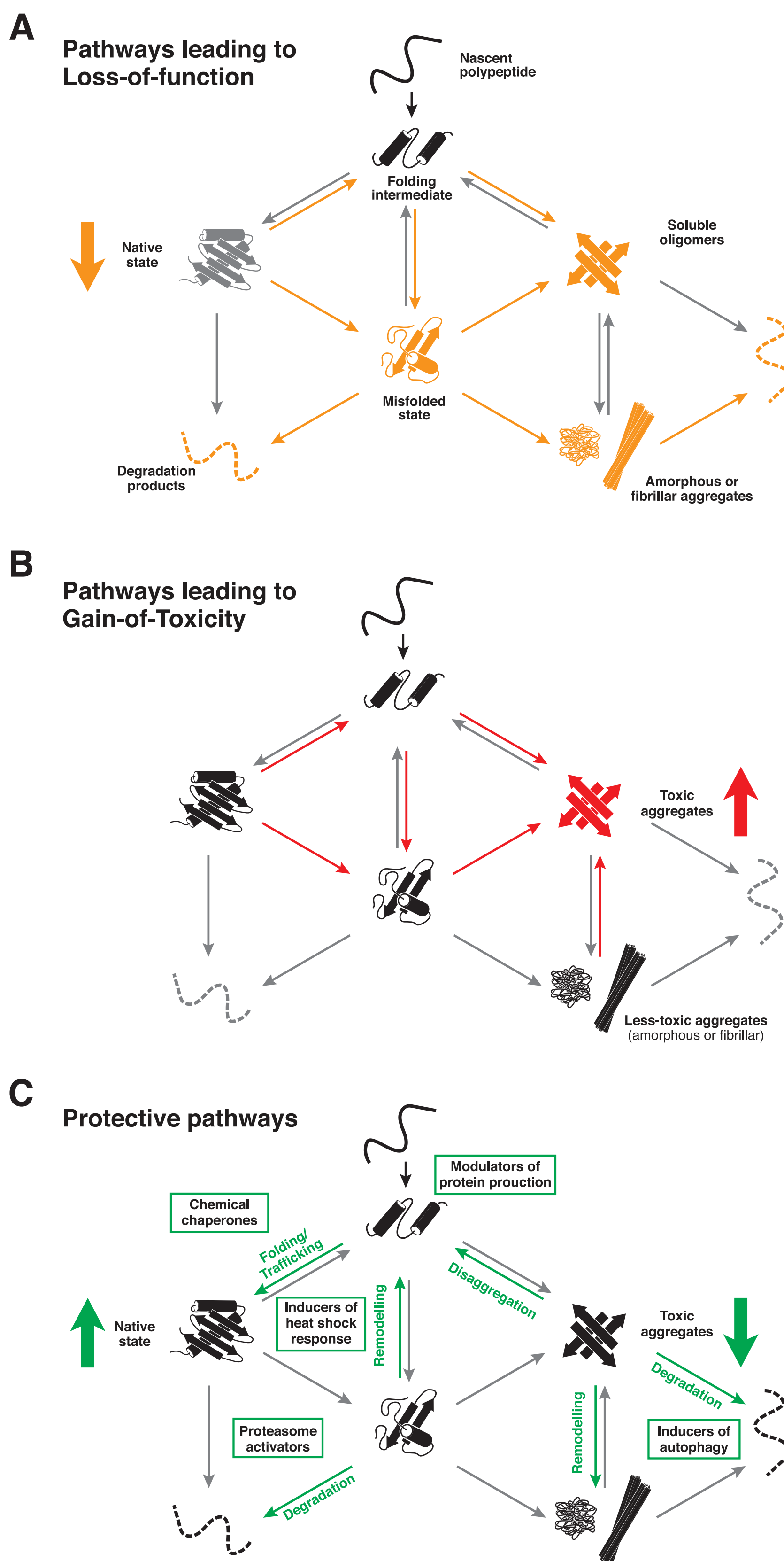
Notably, some phase separated compartments may undergo a transition from liquid-like to more solid structures, characterized by increased molecular packing and reduced fluidity.[215] This transition is influenced by various factors, including molecular concentration, post-translational modifications, and alterations in the local microenvironment. It can significantly impact cellular physiology, aging, and the onset of diseases, as several proteins prone to LLPS, such as RNA-binding proteins (e.g., FUS and TDP43), may undergo a maturation process from liquid-like to fibrillar aggregates associated with neurodegeneration. Mutations in disease proteins can promote this conversion,[216,217] as does the presence of misfolded or aggregated proteins.[113,218–221] In addition to FUS and TDP43, phase separation has also been observed for other proteins connected to protein misfolding diseases, including Tau,[222] α Synuclein[223] and Htt.[224]

Notably, chaperones modulate phase separation and function to maintain a liquid-like, non-fibrillar state. Hsp70s and sHSPs have been reported to alter phase separation of TDP-43 and FUS,[152,225–227] while Hsp40s are additionally involved in the phase separation of polyQ expanded Htt.[135]

As cells age, the proteostasis machinery becomes less efficient at maintaining liquid-like states. This age-dependent decline facilitates the accumulation of potentially pathogenic aggregates.[215,228]

Modules of the PN

The PN can be operationally divided into three different modules or branches that attend to proteins at different stages during their life cycle: Biogenesis, conformational maintenance and degradation. The module for biogenesis contains all the factors necessary for transcription,


A
Pathways leading to Loss-of-function
Nascent polypeptide
Folding intermediate
Native state
Soluble oligomers
Misfolded state
Degradation products
Amorphous or fibrillar aggregates
B
Pathways leading to Gain-of-Toxicity
Toxic aggregates
Less-toxic aggregates (amorphous or fibrillar)
C
Protective pathways
Modulators of protein prouction
Chemical chaperones
Folding/ Trafficking
Disaggregation
Native state
Inducers of heat shock response
Remodelling
Toxic aggregates
Degradation
Proteasome activators
Inducers of autophagy
Remodelling
Degradation

translation, initial folding and transport of a protein to its designated cellular localization. Conformational maintenance comprises the factors needed to maintain proteins in a functional state, after their initial folding has been completed. Finally, the degradation module, which contains by far the most factors, encompasses the machineries for the controlled degradation of functional and defective proteins.

Several cellular membrane compartments and organelles possess specialized quality control networks (and in some cases individual stress response programs), while other quality control capacities may either be present only in rudimentary form or be missing entirely. For example, most proteins are transported into the nucleus after completing folding, so there is no need for nuclear machineries assisting in de novo folding, and the endoplasmic reticulum (ER) generally lacks a protein degradation machinery as proteins designated for degradation are exported to the cytosol in a process called ERAD (ER associated degradation).

Foldase and holdase chaperones

It is now well-established that a substantial fraction of newly synthesized proteins require molecular chaperones for folding and assembly to occur efficiently and at a biologically relevant time scale.[21,30,31] Moreover, many proteins return to chaperones for conformational maintenance throughout their lifespan.[32] Thus, core activities of molecular chaperones encompass their ability to assist in productive de novo folding and refolding of misfolded states (“foldase” function), prevention of off-pathway aggregation (“holdase” function) and the capacity to actively disassemble specific protein aggregates (“disaggregase” function). Specific chaperones can be involved in more than one of these activities.[33] In addition to protein substrates, chaperones may also interact with mRNA, and thereby alter mRNA stability or RNA polymerase III transcription activity.[34,35]

The ATP-independent small heat-shock proteins (sHSPs) are considered archetypical “holdase” chaperones. They mitigate aggregation by binding non-native proteins through hydrophobic interactions.[36,37] On the other hand, by forming oligomeric complexes, sHSPs can also sequester proteins into insoluble deposits, a function that may reduce pressure on the PN under stress conditions and in aging.[38,39] Refolding of sHSP-bound client proteins may occur in cooperation with ATP-dependent Hsp70 chaperones,[40] which bind and release hydrophobic chain segments of non-native polypeptides in an ATP-regulated reaction cycle.[41] This process, which also underlies the function of Hsp70s in co-translational folding, is intri-

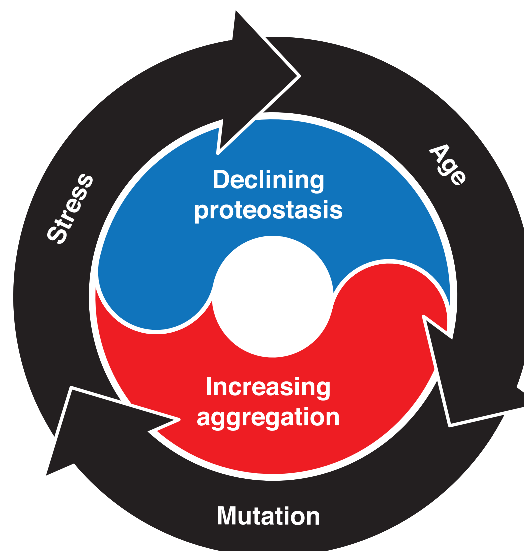


Figure 2. Feed-forward loop between proteostasis decline and increased aggregation. A reduced capacity of the PN increases the risk of protein aggregation. On the other hand, protein aggregates titrate PN components, and reduce the available proteostasis capacity. Both aspects may initiate a feed-forward loop that can lead to a collapse of proteostasis. Disease-causing mutations have been observed that either increase the aggregation tendency of the mutant protein, or reduce parts of the proteostasis machinery. Ageing and stress have also been reported to affect both parts of this loop.

Figure 1. Protein misfolding impairs protein homeostasis due to two different (non-exclusive) mechanisms **A.) Loss-of-function toxicity.** This mode of toxicity is caused by reduced levels of the native, functional state of the misfolded protein. For this aspect of toxicity, the fate of the misfolded protein (degraded, misfolded or aggregated) is not relevant. **B.) Gain-of-toxic function**. This aspect of toxicity is caused by a novel property of the misfolded protein. It is likely that not all forms of a misfolded protein contain these toxic properties to the same degree, and that some misfolded species are more toxic than others. Currently most evidence hints at small oligomeric aggregates as the most toxic forms; however, this does not exclude that larger structures, or misfolded monomers also contribute to toxicity. **C.)** The proteostasis network protects against both types of toxicity, by shifting the equilibrium towards folded proteins and by degrading, disaggregating or remodeling toxic aggregates. Classes of potential drugs that can augment the PN are indicated.

cately regulated by multiple co-chaperones, such as Hsp40s and nucleotide exchange factors (NEFs).[23] Hsp70s also contribute to protein disaggregation, either in cooperation with Hsp40/NEFs[42] or in conjunction with AAA+ ATPase chaperones, such as Hsp104 in fungi[43] and VCP in mammals[44] (see Box: Disaggregases). Furthermore, the Hsp70 system cooperates with the Hsp90 chaperone system and the cylindrical chaperonin complexes (TRiC/CCT) in various folding pathways. Hsp90 and its multiple co-factors play a pivotal role in the folding and regulation of numerous conformationally dynamic proteins, including kinases and other signaling molecules.[45]

Box: Disaggregation

Hsp100 chaperones are a group of specialized chaperones of the AAA+ family in bacteria, yeast, and plants that have the ability to resolve amyloid-like aggregates.[42,191] However, direct homologues of these hexameric disaggregases have not been identified in mammalian cells. Instead, disaggregation in higher eukaryotes is mainly attributed to the Hsp70 chaperone machinery.[192–195] The human Hsp70-Hsp40-Hsp110 chaperone system efficiently dissociates Tau, α Synuclein and Htt fibrils in vitro[196–199] independent of AAA+ disaggregases that cooperate with the Hsp70 system in yeast and bacteria to achieve disaggregation.[191]

Recent findings have added the AAA+ ATPase VCP (also known as P97, Cdc48) as a new factor mediating protein disaggregation. VCP has been shown to disaggregate amyloid fibrils of Tau and mutations in VCP have been shown to be the cause of a form of vacuolar tauopathy with Tau aggregates.[44,200] VCP is distinct from Hsp104 in that it requires the target aggregate to be ubiquitylated, a critical element of control to ensure specificity and avoid dissolution of functional protein assemblies.[201] Moreover, aggregate ubiquitylation ensures that disaggregation by VCP is coupled to degradation by the 26S proteasome. Interestingly, the proteasome can also fragment fibrils in vitro in an ATP dependent manner,[202] again connecting a potential disaggregase with degradation by the ubiquitin proteasome system. Recently, the E3 sumo/ubiquitin-protein ligase TRIM11 has also been reported to dissociate aggregates in vitro,[49,203] adding to a growing number of disaggregase factors.

Fragmentation of amyloid fibrils can result in the formation of seeding competent aggregate species,[44,196,204] and thus coupling disaggregation to degradation might have evolved in multicellular organisms to prevent cell to cell spreading of aggregates. An alternative approach to preventing the generation of seeding competent fibril fragments is the removal of monomeric units from the ends of the fibrils.[197]

Non-human AAA+ ATPases with augmented disaggregase activity and higher specificity for specific disease associated aggregates are currently being developed with the aim to reverse pathogenic protein aggregation.[205–207] Boosting cellular aggregate clearance, perhaps in combination with proteasome activation,[108] may offer a potential therapeutic strategy as long as the production of seeding competent species can be controlled.

Ubiquitin proteasome and autophagy systems

The maintenance of proteostasis requires that protein synthesis and degradation are in balance. Efficient removal of terminally misfolded proteins relies on the ubiquitin–proteasome system (UPS), encompassing over 1,400 proteins in human cells, and the autophagosomal/lysosomal system with around 1,000 components [https://www.proteostasisconsortium.com/pn-annotation/]. Proteasome complexes are localized in the cytosol and nucleus. Proteins of the ER that are destined for degradation need to be retrotranslocated to the cytosol to access the proteasome.[46] Specific components of the UPS functionally cooperate with chaperone machinery. For example, while the cochaperone and ubiquitin ligase, CHIP, can bind non-native proteins and prevent their aggregation,[47] it also forms complexes with Hsp70 and Hsp90, thereby facilitating the ubiquitylation of conformationally defective client proteins.[48] Some ubiquitin ligases, such as TRIM11, have chaperone-like activity and are able to increase the solubility of aggregation-prone proteins for efficient degradation, as reported for the AD protein Tau.[49] Notably, proteins must undergo unfolding before proteasomal degradation, a process mediated by the AAA+ ATPase components of the proteasome complex. Proteins must generally be delivered to the proteasome in a soluble, non-aggregated state, and aggregates may need to be actively disassembled by chaperone machinery to enable degradation via the UPS.[44,50]

Larger protein aggregates and insoluble inclusions, which resist dissociation, can be eliminated through autophagy and subsequent lysosomal degradation, constituting the other major clearance pathway for conformationally aberrant proteins. Autophagy entails the engulfment of material (including whole organelles) within a double-membrane vesicle, known as the autophagosome, which subsequently fuses with the lysosome. In contrast to non-selective autophagy of bulk cytoplasm, aggregates are subject to selective autophagy.[51] The Hsp70 chaperone system plays also a crucial role in this process, as the ubiquitylation of target proteins by STUB1/CHIP and the recruitment of the autophagic ubiquitin adaptor, p62, is facilitated by the Hsp70 co-factor, Bag-3.[52,53] Alternatively, Bag-3 may facilitate selective autophagy independent of substrate ubiquitylation.[54] Additionally, there is evidence suggesting that protein aggregates may initially be actively concentrated within aggresomes[55] or juxtanuclear quality control compartments (JUNQs)[56] via cytoskeleton-based transport processes, followed by recruitment of autophagic machinery. Aggresome and JUNQ formation are believed to provide a mechanism for the sequestration of aggregated proteins into a non-toxic storage form until adequate capacity for degradation

becomes available. Recent evidence suggests that for neurodegenerative disease proteins with expanded polyglutamine (polyQ) sequences, which can form fibrillar or amorphous aggregates, the latter are preferred substrates of autophagy, whereas fibrils are not efficiently encapsulated by autophagosomes[229]. Some misfolded protein species are transported directly to lysosomes by cytosolic Hsp70, a process involving the recognition of a specific peptide motif, KFERQ, found in numerous proteins.[57]

The ageing proteostasis network

Age represents the primary risk factor for an array of disorders associated with protein aggregation, notably AD, Parkinson's disease (PD), Huntington's disease (HD), and various other degenerative conditions.[58]

The age-dependent failure of cells to maintain a functional proteome is regarded as a major driver of age-related cellular dysfunction and degenerative diseases,[32,59] and accordingly proteostasis dysfunction is now being recognized as one of the main hallmarks of ageing.[60] The biological reasons behind this decline are complex, and while its exact causes remain to be established, the lack of evolutionary pressure for proteome maintenance once organisms have produced progeny and passed their genome to the next generation is likely playing a role. The "disposable-soma theory" posits that organisms allocate more resources to propagating the germline than to preserving the integrity of the somatic proteome.[61] This theory finds support in observations that proteostasis in *Caenorhabditis elegans* significantly deteriorates after progeny production.[62] A controlled ageing program is thought to allocate organismal resources to reproduction rather than proteome maintenance,[63] and most genetic manipulations that extend lifespan and improve proteostasis of *C. elegans* are associated with reduced fecundity.

System-wide proteome analyses along the lifespan of *C. elegans* have revealed significant shifts in overall levels and reduced solubility of numerous proteins as the worms age.[38,64–67] This age-dependent proteome remodelling was substantially less pronounced in long-lived worms, which is indicative of an improved capacity of the PN to maintain proteome integrity. In contrast, mice show fewer age-related protein changes,[68] suggesting that mammals invest more resources to maintaining proteome balance as they age.[69]

A characteristic of the ageing proteome is the accumulation of aggregates.[38,64] Studies in *C. elegans* quantifying over 2,000 aggregating proteins found that proteins of low abundance tend to have greater aggregation propensities (i.e. fraction of total that is insoluble) during ageing than abundant proteins.[16,38] Nevertheless, highly abundant proteins were found to predominantly contribute to total aggregate load, despite their greater intrinsic solubility.[38] This supports the view that proteins have been optimized in evolution to maintain solubility at their physiological concentration (before age-dependent proteome dysregulation) but aggregate when exceeding that concentration.[16,70] Apparently, the solubility of a subset of abundant proteins is insufficient to protect them from exceeding their critical soluble concentration during aging. It has been argued that these proteins are "supersaturated" and normally exist at the edge of solubility.[16,70,71]

As organisms age, the PN becomes increasingly burdened by accumulating misfolded proteins and proteins that have been damaged by oxidative stress,[72] which seems to affect predominantly non-dividing, long-lived cells such as neurons.[73,74] Ageing reduces the efficiency of co-translational folding by altering translation elongation speed in *C. elegans* and *Saccharomyces cerevisiae*, leading to increased ribosome collisions that may overwhelm the system of ribosome-associated quality control.[75,76] Analysis of senescent human fibroblasts has shown that the inducibility of the heat shock response is compromised in senescent cells when compared to young cells, and that the coordination between the different branches of the unfolded protein stress response was impaired.[77] While the levels of some chaperones in human brain increase with age, the expression of a critical subnetwork of chaperones, containing many ATP-dependent constituents, decreases.[78]

Once the capacity of the PN drops below a critical threshold, aggregation-prone proteins can no longer be maintained in a soluble state. This threshold is lowered further under additional forms of stress, such as the presence of mutations that structurally destabilize specific proteins rendering them prone to misfolding.[79,80] The additional pressure on the PN promotes protein aggregation in a feed-forward loop (Figure 2).[81,82]

Stem cells and proteostasis maintenance

Notably, stem cells are more resistant to age-dependent proteostasis decline than differentiated cells, and pluripotent stem cells are believed to invest substantially more resources in proteome maintenance compared to differentiated cells.[83,84] Stem cells must continuously replenish their numbers via asymmetric cell division,[85] while keeping their proteome intact. This might be achieved by the retention of damaged and aggregated proteins in one cell, while the other daughter cell inherits a rejuvenated proteome.[86] At the same time, stem cells have to remodel their proteomes as they differentiate into various cell types, highlighting the importance of proteostasis control and rewiring during development.[84,87] Adult hematopoietic, neural, epidermal, and muscle stem cells have relatively low rates of protein production,[88–91] which presum-

ably results in a reduced burden on the protein folding module of the PN. In contrast, embryonic stem cells (ESCs) with their higher rates of proliferation[92] tend to have higher translational rates.[93,94] Human ESCs exhibit elevated levels of proteasome activity for degrading misfolded proteins.[83] Furthermore, human pluripotent stem cells support the efficient assembly of the TRiC/CCT chaperonin complex, apparently by enhancing the expression of one of its eight subunits, CCT8,[95] which limits complex assembly when present in substoichiometric amounts. The higher demand for TRiC/CCT in stem cells may be due to the need for actin, an obligate client protein of TRiC/CCT,[96,97] which is increasingly required in proliferating ESCs for cytoskeletal synthesis and integrity. Experiments in rodents and in fly models suggest that asymmetrical division of at least some types of stem cells might also have a role in maintaining a balanced proteome, with the differentiating cell inheriting the damaged proteins.[98–100] These mechanisms may contribute to the maintenance of stem cells throughout the organismal lifespan. Interestingly, the neural stem cell pool in the brain of adult mice comprises quiescent and activated populations with differences in their proteostasis networks. While activated stem cells have active proteasomes, quiescent stem cells were recently shown to rely on large lysosomes for aggregate removal. Lysosomal damage accrued during ageing may thus reduce the ability of quiescent cells to dispose of aggregates and to reactivate.[99]

Neurodegenerative diseases and protein aggregation

Aggregate-deposition in diseases, including the major age-dependent neurodegenerative disorders, is typically associated with a gain of toxic function of the aggregate. This “dominant” mechanism of cellular pathology differs from recessive loss-of-function diseases like cystic fibrosis, where specific proteins are rendered nonfunctional due to mutations and are targeted for degradation. While the connection between proteostasis decline and aggregation is evident in most major degenerative diseases, the study of pathological polyglutamine (polyQ) proteins in various model systems has provided compelling evidence for the critical role of age-related proteostasis deterioration in disease manifestation.[80]

Unlike most other aggregate-related disorders, HD and other polyQ-expansion diseases follow a dominant inheritance pattern. The clear correlation between the length of the polyQ repeat and its propensity for aggregation, along with an inverse correlation with the age of disease onset, has significantly aided our current understanding of the relationship between available proteostasis capacity and age-dependent disease manifestation: A higher PN capacity is required to prevent the aggregation of long polyQ repeats and, consequently, disease manifestation occurs earlier in life, and indeed, pharmacological activation of the heat shock response (HSR) improves HD-related phenotypes in mouse models of HD.[101] Over time however, HSF1 binding to stress-dependent promoters and HSR induction decreases, limiting the long-term beneficial effects of this treatment.[101] While permanent activation of the general HSR seems to be unachievable, overexpression or activation of various elements of the PN has yielded positive effects. This includes chaperones of the small heat shock protein family,[102] Hsp70/Hsp40 family members[103,104] and the chaperonin TRiC,[105,106] as well as activation of autophagy[107] or the 26S proteasome.[108]

Neurodegenerative diseases are characterized by neuronal dysfunction and cell death, primarily triggered by a subset of toxic aggregate species of specific disease proteins, such as α-Synuclein in PD, Tau in AD and various tauopathies, Huntingtin (Htt) in HD, and SOD1 in certain forms of amyotrophic lateral sclerosis (ALS).[109,110] Importantly, aggregates of heterologous and even artificial proteins are also toxic, suggesting that gain-of-toxic function, not loss-of function is responsible for at least part of the cytotoxicity observed.[111–113] The toxic aggregate species of these proteins are thought to include diffusible oligomeric forms with disordered fibrillar topology and exposed hydrophobic amino acid residues on unpaired beta-strands. Besides interacting with lipid membranes, these soluble aggregates have a high propensity to engage in aberrant interactions with other proteins, eventually resulting in their sequestration in insoluble aggregate deposits. Numerous endogenous proteins, often newly synthesized or featuring extensive disordered regions, as well as specific chaperones and proteasomes, have been found associated with these aggregates.[111,112,114–117] Notably, mounting evidence suggests that sequestering oligomers into large insoluble deposits may offer relative protection,[118] presumably by reducing the interactive, solvent-exposed surface area of the aggregates. However, the chronic presence of large aggregate inclusions is unlikely to be entirely benign, as they cause the displacement and alteration of cellular membrane structures.[119]

Mechanisms underlying proteostasis disruption

Studies in cellular as well as organismal models have shed light on how the chronic production of misfolded and aggregated proteins compromises core functions of the PN, including the cell’s capacity to facilitate protein folding,[79,80] and the clearance of misfolded proteins.[81,120] In healthy cells various interlinked stress response pathways are triggered when misfolded proteins accumulate, including the cytosolic HSR,[121] the unfolded protein response pathways (UPRs) of the ER[122,123] and

mitochondria,[124] the integrated stress response (ISR)[125] and additional networks governing inflammation, and oxidative stress responses. The cytosolic heat shock response is activated when misfolded proteins displace chaperones from the transcription factor heat-shock transcription factor 1 (HSF-1), thereby enabling HSF-1 to inducing the production of stress-inducible chaperones (heat shock proteins).[126,127] However, this response to conformational stress by upregulating PN machinery is inhibited in the presence of aggregation-prone proteins.[111] In yeast, shutdown of rRNA synthesis can also lead to the accumulation of aggregation-prone ribosomal proteins that are not bound to rRNA (orphan ribosomal proteins) and that trigger the localization of chaperones to the nucleolar periphery.[128]

Although the precise manner in which aberrant proteins overburden the PN remains to be investigated in more detail, multiple lines of evidence indicate that aggregated proteins can sequester factors involved in mounting a successful stress response[111,129,130] as well as specific PN components, including chaperones,[116,120] components of the UPS,[117,131] and transport factors,[112,132] rendering them inaccessible to other clients.

The proteins primarily affected by this chaperone titration are members of the 'metastable proteome,' a group of structurally dynamic proteins that require constant chaperone surveillance. Notably, while the acute accumulation of misfolded proteins under stress conditions, such as heat stress, triggers the rapid activation of cytosolic and organellar stress response pathways to restore proteome equilibrium,[126,133] this compensatory response fails with age. As shown in *C. elegans*, repression of the HSR occurs due to an increase in H3K27me3 marks at stress gene loci, resulting in a repressed chromatin state that suppresses transcription initiation in response to stress.[63] Additionally, the chronic accumulation of aberrant protein species, as is characteristic of various diseases and during aging, leads to an inefficient 'maladaptive' stress response.[134] Under such conditions, key components of stress signaling pathways are thought to lose functionality. For instance, the transcription factor NF-Y, which participates in Hsp70 expression, becomes sequestered by polyQ aggregates.[129] PolyQ aggregates also fail to induce the HSF-1 mediated heat shock response, a block that can be overcome, however, by elevating the levels of certain Hsp40 chaperones (Sis1 in yeast and DnajB6 in mammalian cells).[135] These Hsp40s allow Hsp70, a negative regulator of HSF-1, to bind the aggregated polyQ proteins, allowing its displacement from HSF-1 for activation.[135] HSF-1 itself is a metastable protein that can undergo condensation and phase transition,[136] and whose levels are finely regulated by post-translational modifications, chaperones and proteasomal degradation.[133,137,138]

The presence of ubiquitin within the inclusions of virtually all neurodegenerative disease proteins suggests that these proteins evade degradation when they accumulate to levels surpassing proteasomal capacity. A mismatch between aberrant protein species destined for degradation and available proteasome capacity may help to explain why aging is a prominent risk factor for protein aggregation. Conversely, evidence suggests that aggregation is not merely a consequence of malfunction of the UPS but can also be its cause. This viewpoint gains support from findings demonstrating that the expression of structurally unrelated aggregation-prone proteins impedes the proteasomal degradation of other proteins. Noteworthy examples include polyQ expansion proteins,[81,139] the disease-linked prion protein PrpSc,[140] and multiple proteins associated with ALS.[117,141,142] For ALS it has been proposed that aggregates engage with the proteasome but resist unfolding, effectively 'clogging' the system and hindering the entry of other substrates.[117,142] However, in the case of polyQ proteins, in vitro experiments have shown that the polyQ expansion sequence does not inhibit proteasome function directly, as efficient degradation is observed when the proteins are targeted to the proteasome via N- or C-terminal degradation signals.[120,143,144] Thus, indirect effects contribute to the observed accumulation of ubiquitinated proteins, most likely caused by the sequestration and functional depletion of essential PN components. For example, expression of a polyQ-expanded Htt exon 1 fragment was found to stabilize terminally misfolded proteins that would otherwise undergo rapid degradation via the UPS[120] or lead to the aggregation of metastable proteins.[79,145] The finding that there is limited overlap between protein subsets that alter solubility in response to different forms of proteostatic stress may suggest the existence of specific PN modules that respond to different kinds of perturbations.[146]

Chaperone sequestration by aggregation-prone proteins likely contributes to cellular pathology in several neurodegenerative diseases. Aggregates of mutant SOD1 or of C9orf72 dipeptide repeat proteins, linked to ALS, sequester chaperones of the Hsp70 family and their cofactors,[147,148] and similar findings have been reported for engineered beta-sheet proteins forming amyloid-like inclusions.[111,112]

Sustaining the solubility of chronically expressed mutant proteins, such as polyQ-expanded Htt, diverts significant PN resources. Expression of mutant Htt has been shown to disrupt the folding and conformational maintenance of endogenous or exogenously expressed metastable proteins[80,120,145] and interferes with specific chaperone functions, such as Hsc70 dependent clathrin-mediated endocytosis.[116] Moreover, as shown recently, expression of mutant Htt can cause the ubiquitination of chaperones themselves.[149]

The robust and highly interconnected and redundant nature of the PN allows affected cells to withstand the adverse effects of aberrant protein species for extended periods, sometimes spanning decades in humans, leading to late disease onset, even when mutant proteins are expressed throughout lifetime, as in the case of the polyQ expansion diseases.

Mutations in PN Components as Drivers of Neurodegenerative Diseases

The existence of familial forms of neurodegeneration linked to mutations in key components of all branches of the PN highlights the pivotal role of the proteostasis system in the pathogenesis of aggregate-deposition disorders. For example, predisposing mutations for the motor neuron disease ALS have been found in several PN factors, including ubiquilin-2, a protein facilitating the recruitment of proteasome complexes to ubiquitylated proteins[150]; sequestosome-1 (p62), a ubiquitin-binding protein essential for autophagy-mediated clearance of aggregates[151]; HSPB1, a small heat shock protein[152,153]; PDIA1 and PDIA3/ERp57, protein disulfide isomerases involved in ER proteostasis,[154] and VCP (p97/Cdc48), an AAA+ ATPase involved in ER-associated degradation (ERAD)[155] and disaggregation of Tau fibrils.[44] The protein encoded by the C9orf72 plays a role in the initiation of autophagy, and its mutation, the most frequent genetic cause of ALS and frontotemporal dementia,[156,157] not only results in the synthesis of aggregation-prone repeat proteins, but also in a loss of function.[158]

Early-onset PD is attributed to loss-of-function mutations in the ubiquitin ligase PARKIN and the PARKIN-related kinase PINK1.[159,160] PARKIN and PINK1 functionally cooperate in the ubiquitylation and selective autophagy of damaged mitochondria, a process known as mitophagy.[161] Dysregulation of this pathway results in the accumulation of dysfunctional mitochondria, disruptions in calcium homeostasis, and heightened oxidative stress. Mutations in the gene encoding the extracellular chaperone Clusterin are a major risk factor for late-onset AD.[162,163] Interestingly, Clusterin may have both positive and negative effects on disease progression, as experiments in cell culture have shown that the chaperone can stabilize Tau aggregates in a form highly competent for seeding new aggregates upon uptake by naïve cells.[164,165] Marinesco-Sjögren syndrome, a rare autosomal-recessive disorder characterized by cerebellar ataxia, can be attributed to loss-of-function mutations affecting the HSPA5 cochaperone SIL1. SIL1 plays a crucial role in protein translocation and folding within the ER.[166,167] Furthermore, mutations involving the mitochondrial chaperonin Hsp60 are implicated in autosomal-dominant spastic paraplegia[168] and an autosomal-recessive neurodegenerative disorder linked to brain hypomyelination and leukodystrophy.[169]

Pharmacological Strategies for Enhancing Proteostasis

The use of so-called chemical chaperones has been successful in stabilizing the folded and assembled state of specific proteins, as has been demonstrated for transthyretin amyloidosis (ATTR), cystic fibrosis (CF) and other protein misfolding diseases.[170] In ATTR, stabilization of transthyretin tetramers by Tafamidis, which binds transthyretin specifically, has been demonstrated to slow disease progression in patients.[171] Similarly, chemical chaperones can improve cell surface localization of some CFTR mutants.[172,173]

While these approaches are disease protein-specific, modulation of PN capacity by pharmacologic activation of the major transcriptional stress response pathways might provide more general benefits. Various studies have shown the positive effects of inducing the cytosolic stress response using small-molecule compounds or overexpression of components of the Hsp70 system in cells expressing different aggregation-prone proteins.[135,174–176] Such interventions prevent the formation of toxic aggregates while promoting the generation of presumably less toxic inclusion bodies[103,177] or phase separated assemblies (see Box Phase Separation).

Enhancing ER folding capacity through activation of the ER stress response can improve the secretion of specific disease proteins, including mutant α1-antitrypsin, and ameliorate lysosomal enzyme deficiencies. The induction of the ER stress factor XBP1s has demonstrated potential in preventing amyloid-beta neurotoxicity in models of AD. Adjusting protein production rates to levels manageable by available chaperones can be achieved by modulators of the phosphorylation status of the translation initiation factor subunit eIF2α, including small molecule drugs like Guanabenz,[178] Sephin[179] or ISRIB.[125] Alternatively, increasing proteolytic capacity offers another approach to sustaining proteostasis, which can be realized through induction of autophagy[180] or the proteasome,[181,182] or by inhibiting specific deubiquitinating enzymes, thereby accelerating the clearance of misfolded proteins by the UPS.[183] In the case of glycosylated proteins, like α1-antitrypsin, modulation of N-glycans might be another approach to alter proper folding, quality control and trafficking of mutant proteins.[184]

Beyond mitigating the toxic effects of aggregating disease-associated proteins, augmenting proteostasis capacity has been linked to extended lifespan and the preservation of responsiveness to acute stress in model organisms.[185] Conversely,

the presence of aggregates over prolonged periods can dampen the ability of cells to respond adequately to stress, suggesting that protein aggregation plays a significant role in the aging process. While small-molecule activators of the stress response have shown efficacy early in disease, their effectiveness may diminish with disease progression and aging.[101] By temporarily downregulating general translation, the cytosolic and ER stress response pathways may be most suitable in combatting acute conformational stress, but this strategy is less effective in counteracting chronic protein misfolding underlying disease. For example, chronic ER stress-induced down-regulation of translation can be particularly detrimental to neuronal cells, which heavily rely on ongoing translation for functionality.[186,187]

Understanding the mechanisms through which protein aggregation disrupts stress-response pathways, thereby undermining cellular defenses, remains pivotal in devising therapeutic strategies based on PN modulation. In any case, pharmacological interventions aimed at enhancing proteostasis are likely to be most effective when initiated early in the disease process, before severe cellular dysfunction becomes manifest. These interventions must be well controlled, however, as upregulation of stress response pathways, such as the cytosolic stress response and the UPR^{ER}, can also support tumor growth, considering that cancer cells may depend on signaling proteins with conformationally destabilizing mutations.[188–190]

Conclusion

In the 15 years since the term proteostasis was coined[1] we have witnessed important progress in cellular biology, spotlighting the critical role of the PN in health and disease. We now recognize proteostasis as a fundamental mechanism involved not only in the folding, conformational maintenance and turnover of every protein in the cell, but also controlling the disruptive impact of chronic protein misfolding and toxic aggregation that drives the onset and progression of numerous neurodegenerative pathologies and other disorders. At the heart of this lies the intricate interconnection between the chaperone machineries governing protein folding and the systems of protein degradation. When specific components of the PN are compromised, such as through their sequestration by aggregates, far-reaching consequences reverberate throughout the cellular landscape. This sets in motion a self-perpetuating cycle that amplifies proteome imbalances, ultimately culminating in the collapse of proteostasis and the demise of the cell.

The gradual accumulation of proteome damage within postmitotic tissues, coupled with the age-associated decline in proteostasis capacity and the perturbation of stress-response pathways, offers a compelling explanation for aging as the predominant risk factor in aggregate-deposition diseases. In light of these insights, the quest for effective pharmacological strategies to augment rate limiting PN components emerges as a promising avenue for therapeutic intervention. However, the achievement of this objective hinges on our ability to gain insights into the organizational structure and hierarchical arrangement of the PN, as well as a comprehensive understanding of the signaling pathways involved in its regulation.

The PN is able to maintain a healthy proteome for decades, before age related protein aggregation becomes manifest. This is also true in many heritable forms of protein misfolding diseases, even though here the toxic mutant protein is present throughout the lifetime of the affected individuals. The genetic information, and therefore the blueprint for the production of all PN components necessary to maintain a healthy proteome is not lost in aged individuals. It is currently not clear if there is a universal pathway that can rejuvenate the PN in a whole organism, or if approaches targeting specific cell types or mutations are more promising. Finding ways to reestablish or maintain a youthful PN capacity without unwanted consequences, such as the stabilization of oncogenic mutations, poses a major challenge. Future research will hopefully bring us closer to reaching this goal.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used GPT-3.5 in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Mark S. Hipp: Writing – review & editing, Writing – original draft. **F. Ulrich Hartl:** Writing – review & editing, Writing – original draft.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Research in F.U.H's laboratory on neurodegenerative aggregation diseases is supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy – ID 390857198) and by the joint efforts of The Michael J. Fox Foundation for Parkinson's Research (MJFF) and the Aligning Science Across Parkinson's (ASAP) initiative. MJFF administers the grant ASAP-000282 on behalf of ASAP and itself. Research in M.S.H's laboratory on neurodegenerative aggregation diseases is supported by Alzheimer Nederland (grant WE.03-2020-12) and the research pool of the Carl von Ossietzky Universität Oldenburg (FP2023-086).

Received 8 February 2024;
Accepted 13 May 2024;
Available online xxxx

Keywords:
proteostasis;
protein aggregation;
neurodegenerative disease;
protein folding

References

1. Balch, W.E., Morimoto, R.I., Dillin, A., Kelly, J.W., (2008). Adapting proteostasis for disease intervention. *Science* **319**, 916–919.
2. Lodish, H.F., (1976). Translational control of protein synthesis. *Annu. Rev. Biochem* **45**, 39–72.
3. Varshavsky, A., (2012). The ubiquitin system, an immense realm. *Annu. Rev. Biochem* **81**, 167–176.
4. Ciechanover, A., (2017). Intracellular protein degradation: from a vague idea thru the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting. *Best Pract. Res. Clin. Haematol.* **30**, 341–355.
5. Dikic, I., (2017). Proteasomal and autophagic degradation systems. *Annu. Rev. Biochem* **86**, 193–224.
6. Eguchi, Y., Makanae, K., Hasunuma, T., Ishibashi, Y., Kito, K., Moriya, H., (2018). Estimating the protein burden limit of yeast cells by measuring the expression limits of glycolytic proteins. *Elife*, 7.
7. Pines, J., (2006). Mitosis: a matter of getting rid of the right protein at the right time. *Trends Cell Biol.* **16**, 55–63.
8. Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S., Igarashi, K., Tamura, T., et al., (1992). Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. *Nature* **360**, 597–599.
9. Faust, J.R., Luskey, K.L., Chin, D.J., Goldstein, J.L., Brown, M.S., (1982). Regulation of synthesis and degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase by low density lipoprotein and 25-hydroxycholesterol in UT-1 cells. *PNAS* **79**, 5205–5209.
10. Ross, C.A., Poirier, M.A., (2004). Protein aggregation and neurodegenerative disease. *Nature Med.*, S10–S17.
11. Scheibel, T., Buchner, J., (2006). Protein aggregation as a cause for disease. *Handb. Exp. Pharmacol.* **199–219**
12. Chiti, F., Dobson, C.M., (2017). Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. *Annu. Rev. Biochem* **86**, 27–68.
13. Winklhofer, K.F., Tatzelt, J., Haass, C., (2008). The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. *EMBO J.* **27**, 336–349.
14. Iadanza, M.G., Jackson, M.P., Hewitt, E.W., Ranson, N. A., Radford, S.E., (2018). A new era for understanding amyloid structures and disease. *Nature Rev. Mol. Cell Biol.* **19**, 755–773.
15. Tartaglia, G.G., Pechmann, S., Dobson, C.M., Vendruscolo, M., (2007). Life on the edge: a link between gene expression levels and aggregation rates of human proteins. *Trends Biochem. Sci* **32**, 204–206.
16. Vecchi, G., Sormanni, P., Mannini, B., Vandelli, A., Tartaglia, G.G., Dobson, C.M., et al., (2020). Proteome-wide observation of the phenomenon of life on the edge of solubility. *PNAS* **117**, 1015–1020.
17. Brown, G.C., (1991). Total cell protein concentration as an evolutionary constraint on the metabolic control distribution in cells. *J. Theor. Biol.* **153**, 195–203.
18. Ellis, R.J., Minton, A.P., (2006). Protein aggregation in crowded environments. *Biol. Chem.* **387**, 485–497.
19. van der Lee, R., Buljan, M., Lang, B., Weatheritt, R.J., Daughdrill, G.W., Dunker, A.K., et al., (2014). Classification of intrinsically disordered regions and proteins. *Chem. Rev.* **114**, 6589–6631.
20. Tsoi, P.S., Quan, M.D., Ferreon, J.C., Ferreon, A.C.M., (2023). Aggregation of disordered proteins associated with neurodegeneration. *Int. J. Mol. Sci.* **24**
21. Balchin, D., Hayer-Hartl, M., Hartl, F.U., (2020). Recent advances in understanding catalysis of protein folding by molecular chaperones. *FEBS Letter* **594**, 2770–2781.
22. Schopf, F.H., Biebl, M.M., Buchner, J., (2017). The HSP90 chaperone machinery. *Nature Rev. Mol. Cell Biol.* **18**, 345–360.
23. Kampinga, H.H., Craig, E.A., (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nature Rev. Mol. Cell Biol.* **11**, 579–592.
24. Gamerdinger, M., Deuerling, E., (2023). Cotranslational sorting and processing of newly synthesized proteins in eukaryotes. *Trends Biochem. Sci*.
25. Mogk, A., Ruger-Herreros, C., Bukau, B., (2019). Cellular functions and mechanisms of action of small heat shock proteins. *Annu. Rev. Microbiol.* **73**, 89–110.
26. Horovitz, A., Reingewertz, T.H., Cuellar, J., Valpuesta, J. M., (2022). Chaperonin mechanisms: multiple and (mis) understood? *Annu. Rev. Biophys.* **51**, 115–133.
27. Landreh, M., Sawaya, M.R., Hipp, M.S., Eisenberg, D.S., Wuthrich, K., Hartl, F.U., (2016). The formation, function and regulation of amyloids: insights from structural biology. *J. Intern. Med.* **280**, 164–176.
28. Sawaya, M.R., Hughes, M.P., Rodriguez, J.A., Riek, R., Eisenberg, D.S., (2021). The expanding amyloid family:

structure, stability, function, and pathogenesis. *Cell* **184**, 4857–4873.
29. Louros, N., Schymkowitz, J., Rousseau, F., (2023). Mechanisms and pathology of protein misfolding and aggregation. *Nature Rev. Mol. Cell Biol.* **24**, 912–933.
30. Frydman, J., (2001). Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu. Rev. Biochem* **70**, 603–647.
31. Kim, Y.E., Hipp, M.S., Bracher, A., Hayer-Hartl, M., Hartl, F.U., (2013). Molecular chaperone functions in protein folding and proteostasis. *Annu. Rev. Biochem* **82**, 323–355.
32. Hipp, M.S., Kasturi, P., Hartl, F.U., (2019). The proteostasis network and its decline in ageing. *Nature Rev. Mol. Cell Biol.* **20**, 421–435.
33. Rutledge, B.S., Choy, W.Y., Duennwald, M.L., (2022). Folding or holding?-Hsp70 and Hsp90 chaperoning of misfolded proteins in neurodegenerative disease. *J. Biol. Chem.* **298**, 101905
34. Leone, S., Srivastava, A., Herrero-Ruiz, A., Hummel, B., Tittel, L., Campalastri, R., et al., (2024). HSP70 binds to specific non-coding RNA and regulates human RNA polymerase III. *Mol. Cell*.
35. Georgellis, D., Sohlberg, B., Hartl, F.U., von Gabain, A., (1995). Identification of GroEL as a constituent of an mRNA-protection complex in Escherichia coli. *Mol. Microbiol.* **16**, 1259–1268.
36. Haslbeck, M., Weinkauf, S., Buchner, J., (2019). Small heat shock proteins: Simplicity meets complexity. *J. Biol. Chem.* **294**, 2121–2132.
37. Reinle, K., Mogk, A., Bukau, B., (2022). The diverse functions of small heat shock proteins in the proteostasis network. *J. Mol. Biol.* **434**, 167157
38. Walther, D.M., Kasturi, P., Zheng, M., Pinkert, S., Vecchi, G., Ciryam, P., et al., (2015). Widespread proteome remodeling and aggregation in aging C. elegans. *Cell* **161**, 919–932.
39. Shrivastava, A., Sandhof, C.A., Reinle, K., Jawed, A., Ruger-Herreros, C., Schwarz, D., et al., (2022). The cytoprotective sequestration activity of small heat shock proteins is evolutionarily conserved. *J. Cell Biol.* **221**
40. Zwirowski, S., Klosowska, A., Obuchowski, I., Nillegoda, N.B., Pirog, A., Zietkiewicz, S., et al., (2017). Hsp70 displaces small heat shock proteins from aggregates to initiate protein refolding. *EMBO J.* **36**, 783–796.
41. Clerico, E.M., Meng, W., Pozhidaeva, A., Bhasne, K., Petridis, C., Gierasch, L.M., (2019). Hsp70 molecular chaperones: multifunctional allosteric holding and unfolding machines. *Biochem. J* **476**, 1653–1677.
42. Mogk, A., Bukau, B., Kampinga, H.H., (2018). Cellular handling of protein aggregates by disaggregation machines. *Mol. Cell* **69**, 214–226.
43. Glover, J.R., Lindquist, S., (1998). Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* **94**, 73–82.
44. Saha, I., Yuste-Checa, P., Da Silva, P.M., Guo, Q., Korner, R., Holthusen, H., et al., (2023). The AAA+ chaperone VCP disaggregates Tau fibrils and generates aggregate seeds in a cellular system. *Nature Commun.* **14**, 560.
45. Moran Luengo, T., Mayer, M.P., Rudiger, S.G.D., (2019). The Hsp70-Hsp90 chaperone cascade in protein folding. *Trends Cell Biol.* **29**, 164–177.
46. Christianson, J.C., Jarosch, E., Sommer, T., (2023). Mechanisms of substrate processing during ER-associated protein degradation. *Nature Rev. Mol. Cell Biol.*.
47. Rosser, M.F., Washburn, E., Muchowski, P.J., Patterson, C., Cyr, D.M., (2007). Chaperone functions of the E3 ubiquitin ligase CHIP. *J. Biol. Chem.* **282**, 22267–22277.
48. Kettern, N., Dreiseidler, M., Tawo, R., Hohfeld, J., (2010). Chaperone-assisted degradation: multiple paths to destruction. *Biol. Chem.* **391**, 481–489.
49. Zhang, Z.Y., Harischandra, D.S., Wang, R., Ghaisas, S., Zhao, J.Y., McMonagle, T.P., et al., (2023). TRIM11 protects against tauopathies and is down-regulated in Alzheimer's disease. *Science* **381**, eadd6696
50. den Brave, F., Cairo, L.V., Jagadeesan, C., Ruger-Herreros, C., Mogk, A., Bukau, B., Jentsch, S., (2020). Chaperone-mediated protein disaggregation triggers proteolytic clearance of intra-nuclear protein inclusions. *Cell Rep.* **31**, 107680
51. Mauthe, M., Kampinga, H.H., Hipp, M.S., Reggiori, F., (2023). Digest it all: the lysosomal turnover of cytoplasmic aggregates. *Trends Biochem. Sci* **48**, 216–228.
52. Tedesco, B., Vendredy, L., Timmerman, V., Poletti, A., (2023). The chaperone-assisted selective autophagy complex dynamics and dysfunctions. *Autophagy* **19**, 1619–1641.
53. Arndt, V., Dick, N., Tawo, R., Dreiseidler, M., Wenzel, D., Hesse, M., et al., (2010). Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr. Biol.* **20**, 143–148.
54. Gamerdinger, M., Kaya, A.M., Wolfrum, U., Clement, A. M., Behl, C., (2011). BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins. *EMBO Rep.* **12**, 149–156.
55. Kopito, R.R., (2000). Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* **10**, 524–530.
56. Kaganovich, D., Kopito, R., Frydman, J., (2008). Misfolded proteins partition between two distinct quality control compartments. *Nature* **454**, 1088–1095.
57. Kaushik, S., Cuervo, A.M., (2018). The coming of age of chaperone-mediated autophagy. *Nature Rev. Mol. Cell Biol.* **19**, 365–381.
58. Dillin, A., Cohen, E., (2011). Ageing and protein aggregation-mediated disorders: from invertebrates to mammals. *Philos. Trans. R. Soc. London B Biol. Sci.* **366**, 94–98.
59. Taylor, R.C., Dillin, A., (2011). Aging as an event of proteostasis collapse. *Cold Spring Harb. Perspect. Biol.* **3**
60. Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., (2023). Hallmarks of aging: an expanding universe. *Cell* **186**, 243–278.
61. Kirkwood, T.B., (1977). Evolution of ageing. *Nature* **270**, 301–304.
62. Sala, A.J., Morimoto, R.I., (2022). Protecting the future: balancing proteostasis for reproduction. *Trends Cell Biol.* **32**, 202–215.
63. Labbadia, J., Morimoto, R.I., (2015). Repression of the heat shock response is a programmed event at the onset of reproduction. *Mol. Cell* **59**, 639–650.
64. David, D.C., Ollikainen, N., Trinidad, J.C., Cary, M.P., Burlingame, A.L., Kenyon, C., (2010). Widespread protein aggregation as an inherent part of aging in C. elegans. *PLoS Biol.* **8**, e1000450

65. Reis-Rodrigues, P., Czerwieniec, G., Peters, T.W., Evani, U.S., Alavez, S., Gaman, E.A., et al., (2012). Proteomic analysis of age-dependent changes in protein solubility identifies genes that modulate lifespan. *Aging Cell* **11**, 120–127.
66. Liang, V., Ullrich, M., Lam, H., Chew, Y.L., Banister, S., Song, X., et al., (2014). Altered proteostasis in aging and heat shock response in C. elegans revealed by analysis of the global and de novo synthesized proteome. *Cell. Mol. Life Sci.* **71**, 3339–3361.
67. Zimmerman, S.M., Hinkson, I.V., Elias, J.E., Kim, S.K., (2015). Reproductive aging drives protein accumulation in the uterus and limits lifespan in C. elegans. *PLoS Genet.* **11**, e1005725
68. Walther, D.M., Mann, M., (2011). Accurate quantification of more than 4000 mouse tissue proteins reveals minimal proteome changes during aging. *Mol. Cell. Proteomics* **10** M110 004523.
69. Ori, A., Toyama, B.H., Harris, M.S., Bock, T., Iskar, M., Bork, P., et al., (2015). Integrated transcriptome and proteome analyses reveal organ-specific proteome deterioration in old rats. *Cell Syst.* **1**, 224–237.
70. Ciryam, P., Kundra, R., Morimoto, R.I., Dobson, C.M., Vendruscolo, M., (2015). Supersaturation is a major driving force for protein aggregation in neurodegenerative diseases. *Trends Pharmacol. Sci.* **36**, 72–77.
71. Ciryam, P., Tartaglia, G.G., Morimoto, R.I., Dobson, C.M., Vendruscolo, M., (2013). Widespread aggregation and neurodegenerative diseases are associated with supersaturated proteins. *Cell Rep.* **5**, 781–790.
72. Powers, E.T., Morimoto, R.I., Dillin, A., Kelly, J.W., Balch, W.E., (2009). Biological and chemical approaches to diseases of proteostasis deficiency. *Annu. Rev. Biochem* **78**, 959–991.
73. Sala, A.J., Bott, L.C., Morimoto, R.I., (2017). Shaping proteostasis at the cellular, tissue, and organismal level. *J. Cell Biol.* **216**, 1231–1241.
74. Kundra, R., Ciryam, P., Morimoto, R.I., Dobson, C.M., Vendruscolo, M., (2017). Protein homeostasis of a metastable subproteome associated with Alzheimer's disease. *PNAS* **114**, E5703–E5711.
75. Stein, K.C., Morales-Polanco, F., van der Lienden, J., Rainbolt, T.K., Frydman, J., (2022). Ageing exacerbates ribosome pausing to disrupt cotranslational proteostasis. *Nature* **601**, 637–642.
76. Muller, M.B.D., Kasturi, P., Jayaraj, G.G., Hartl, F.U., (2023). Mechanisms of readthrough mitigation reveal principles of GCN1-mediated translational quality control. *Cell* **186**, 3227–3244.e20.
77. Sabath, N., Levy-Adam, F., Younis, A., Rozales, K., Meller, A., Hadar, S., et al., (2020). Cellular proteostasis decline in human senescence. *PNAS* **117**, 31902–31913.
78. Brehme, M., Voisine, C., Rolland, T., Wachi, S., Soper, J. H., Zhu, Y., et al., (2014). A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep.* **9**, 1135–1150.
79. Gupta, R., Kasturi, P., Bracher, A., Loew, C., Zheng, M., Villella, A., et al., (2011). Firefly luciferase mutants as sensors of proteome stress. *Nature Methods* **8**, 879–884.
80. Gidalevitz, T., Ben-Zvi, A., Ho, K.H., Brignull, H.R., Morimoto, R.I., (2006). Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* **311**, 1471–1474.
81. Hipp, M.S., Patel, C.N., Bersuker, K., Riley, B.E., Kaiser, S.E., Shaler, T.A., et al., (2012). Indirect inhibition of 26S proteasome activity in a cellular model of Huntington's disease. *J. Cell Biol.* **196**, 573–587.
82. Hipp, M.S., Park, S.H., Hartl, F.U., (2014). Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends Cell Biol.* **24**, 506–514.
83. Vilchez, D., Boyer, L., Morantte, I., Lutz, M., Merkwirth, C., Joyce, D., et al., (2012). Increased proteasome activity in human embryonic stem cells is regulated by PSMD11. *Nature* **489**, 304–308.
84. Vilchez, D., Simic, M.S., Dillin, A., (2014). Proteostasis and aging of stem cells. *Trends Cell Biol.* **24**, 161–170.
85. Knoblich, J.A., (2008). Mechanisms of asymmetric stem cell division. *Cell* **132**, 583–597.
86. Liu, B., Larsson, L., Caballero, A., Hao, X., Oling, D., Grantham, J., Nystrom, T., (2010). The polarisome is required for segregation and retrograde transport of protein aggregates. *Cell* **140**, 257–267.
87. Thiruvalluvan, A., de Mattos, E.P., Brunsting, J.F., Bakels, R., Serlidaki, D., Barazzuol, L., et al., (2020). DNAJB6, a key factor in neuronal sensitivity to amyloidogenesis. *Mol. Cell* **78**, 346–358.e9.
88. Signer, R.A., Magee, J.A., Salic, A., Morrison, S.J., (2014). Haematopoietic stem cells require a highly regulated protein synthesis rate. *Nature* **509**, 49–54.
89. Llorens-Bobadilla, E., Zhao, S., Baser, A., Saiz-Castro, G., Zwadlo, K., Martin-Villalba, A., (2015). Single-cell transcriptomics reveals a population of dormant neural stem cells that become activated upon brain injury. *Cell Stem Cell* **17**, 329–340.
90. Blanco, S., Bandiera, R., Popis, M., Hussain, S., Lombard, P., Aleksic, J., et al., (2016). Stem cell function and stress response are controlled by protein synthesis. *Nature* **534**, 335–340.
91. Zismanov, V., Chichkov, V., Colangelo, V., Jamet, S., Wang, S., Syme, A., et al., (2016). Phosphorylation of eIF2alpha is a translational control mechanism regulating muscle stem cell quiescence and self-renewal. *Cell Stem Cell* **18**, 79–90.
92. Garcia-Prat, L., Martinez-Vicente, M., Perdiguero, E., Ortet, L., Rodriguez-Ubreva, J., Rebollo, E., et al., (2016). Autophagy maintains stemness by preventing senescence. *Nature* **529**, 37–42.
93. Ingolia, N.T., Lareau, L.F., Weissman, J.S., (2011). Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell* **147**, 789–802.
94. You, K.T., Park, J., Kim, V.N., (2015). Role of the small subunit processome in the maintenance of pluripotent stem cells. *Genes Dev.* **29**, 2004–2009.
95. Noormohammadi, A., Khodakarami, A., Gutierrez-Garcia, R., Lee, H.J., Koyuncu, S., Konig, T., et al., (2016). Somatic increase of CCT8 mimics proteostasis of human pluripotent stem cells and extends C. elegans lifespan. *Nature Commun.* **7**, 13649
96. Yam, A.Y., Xia, Y., Lin, H.T., Burlingame, A., Gerstein, M., Frydman, J., (2008). Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. *Nature Struct. Mol. Biol.* **15**, 1255–1262.

97. Balchin, D., Milicic, G., Strauss, M., Hayer-Hartl, M., Hartl, F.U., (2018). Pathway of actin folding directed by the eukaryotic chaperonin TRiC. *Cell* **174**, 1507–1521.e16.
98. Bufalino, M.R., DeVeale, B., van der Kooy, D., (2013). The asymmetric segregation of damaged proteins is stem cell-type dependent. *J. Cell Biol.* **201**, 523–530.
99. Leeman, D.S., Hebestreit, K., Ruetz, T., Webb, A.E., McKay, A., Pollina, E.A., et al., (2018). Lysosome activation clears aggregates and enhances quiescent neural stem cell activation during aging. *Science* **359**, 1277–1283.
100. Moore, D.L., Pilz, G.A., Arauzo-Bravo, M.J., Barral, Y., Jessberger, S., (2015). A mechanism for the segregation of age in mammalian neural stem cells. *Science* **349**, 1334–1338.
101. Labbadia, J., Cunliffe, H., Weiss, A., Katsyuba, E., Sathasivam, K., Seredenina, T., et al., (2011). Altered chromatin architecture underlies progressive impairment of the heat shock response in mouse models of Huntington disease. *J. Clin. Invest.* **121**, 3306–3319.
102. Vos, M.J., Zijlstra, M.P., Kanon, B., van Waarde-Verhagen, M.A., Brunt, E.R., Oosterveld-Hut, H.M., et al., (2010). HSPB7 is the most potent polyQ aggregation suppressor within the HSPB family of molecular chaperones. *Hum. Mol. Genet.* **19**, 4677–4693.
103. Muchowski, P.J., Schaffar, G., Sittler, A., Wanker, E.E., Hayer-Hartl, M.K., Hartl, F.U., (2000). Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *PNAS* **97**, 7841–7846.
104. Hageman, J., Rujano, M.A., van Waarde, M.A., Kakkar, V., Dirks, R.P., Govorukhina, N., et al., (2010). A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. *Mol. Cell* **37**, 355–369.
105. Behrends, C., Langer, C.A., Boteva, R., Bottcher, U.M., Stemp, M.J., Schaffar, G., et al., (2006). Chaperonin TRiC promotes the assembly of polyQ expansion proteins into nontoxic oligomers. *Mol. Cell* **23**, 887–897.
106. Tam, S., Geller, R., Spiess, C., Frydman, J., (2006). The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. *Nature Cell Biol.* **8**, 1155–1162.
107. Sarkar, S., Perlstein, E.O., Imarisio, S., Pineau, S., Cordenier, A., Maglathlin, R.L., et al., (2007). Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nature Chem. Biol.* **3**, 331–338.
108. VerPlank, J.J.S., Tyrkalska, S.D., Fleming, A., Rubinsztein, D.C., Goldberg, A.L., (2020). cGMP via PKG activates 26S proteasomes and enhances degradation of proteins, including ones that cause neurodegenerative diseases. *PNAS* **117**, 14220–14230.
109. Wilson 3rd, D.M., Cookson, M.R., Van Den Bosch, L., Zetterberg, H., Holtzman, D.M., Dewachter, I., (2023). Hallmarks of neurodegenerative diseases. *Cell* **186**, 693–714.
110. Taylor, J.P., Hardy, J., Fischbeck, K.H., (2002). Toxic proteins in neurodegenerative disease. *Science* **296**, 1991–1995.
111. Olzscha, H., Schermann, S.M., Woerner, A.C., Pinkert, S., Hecht, M.H., Tartaglia, G.G., et al., (2011). Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* **144**, 67–78.
112. Woerner, A.C., Frottin, F., Hornburg, D., Feng, L.R., Meissner, F., Patra, M., et al., (2016). Cytoplasmic protein aggregates interfere with nucleocytoplasmic transport of protein and RNA. *Science* **351**, 173–176.
113. Frottin, F., Schueder, F., Tiwary, S., Gupta, R., Korner, R., Schlichthaerle, T., et al., (2019). The nucleolus functions as a phase-separated protein quality control compartment. *Science* **365**, 342–347.
114. Kim, Y.E., Hosp, F., Frottin, F., Ge, H., Mann, M., Hayer-Hartl, M., Hartl, F.U., (2016). Soluble oligomers of polyQ-expanded huntingtin target a multiplicity of key cellular factors. *Mol. Cell* **63**, 951–964.
115. Wear, M.P., Kryndushkin, D., O'Meally, R., Sonnenberg, J.L., Cole, R.N., Shewmaker, F.P., (2015). Proteins with intrinsically disordered domains are preferentially recruited to polyglutamine aggregates. *PLoS One* **10**, e0136362.
116. Yu, A., Shibata, Y., Shah, B., Calamini, B., Lo, D.C., Morimoto, R.I., (2014). Protein aggregation can inhibit clathrin-mediated endocytosis by chaperone competition. *PNAS* **111**, E1481–E1490.
117. Guo, Q., Lehmer, C., Martinez-Sanchez, A., Rudack, T., Beck, F., Hartmann, H., et al., (2018). In situ structure of neuronal C9orf72 Poly-GA aggregates reveals proteasome recruitment. *Cell* **172**, 696–705.e12.
118. Arrasate, M., Mitra, S., Schweitzer, E.S., Segal, M.R., Finkbeiner, S., (2004). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* **431**, 805–810.
119. Bauerlein, F.J.B., Saha, I., Mishra, A., Kalemanov, M., Martinez-Sanchez, A., Klein, R., et al., (2017). In situ architecture and cellular interactions of PolyQ inclusions. *Cell* **171**, 179–187.e10.
120. Park, S.H., Kukushkin, Y., Gupta, R., Chen, T., Konagai, A., Hipp, M.S., et al., (2013). PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. *Cell* **154**, 134–145.
121. Morimoto, R.I., (2011). The heat shock response: systems biology of proteotoxic stress in aging and disease. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 91–99.
122. Karagoz, G.E., Acosta-Alvear, D., Walter, P., (2019). The unfolded protein response: detecting and responding to fluctuations in the protein-folding capacity of the endoplasmic reticulum. *Cold Spring Harb. Perspect. Biol.* **11**
123. Hetz, C., Zhang, K., Kaufman, R.J., (2020). Mechanisms, regulation and functions of the unfolded protein response. *Nature Rev. Mol. Cell Biol.* **21**, 421–438.
124. Naresh, N.U., Haynes, C.M., (2019). Signaling and regulation of the mitochondrial unfolded protein response. *Cold Spring Harb. Perspect. Biol.* **11**
125. Costa-Mattioli, M., Walter, P., (2020). The integrated stress response: from mechanism to disease. *Science*, 368.
126. Pincus, D., (2020). Regulation of Hsf1 and the heat shock response. *Adv. Exp. Med. Biol.* **1243**, 41–50.
127. Morimoto, R.I., (1998). Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev.* **12**, 3788–3796.

128. Ali, A., Garde, R., Schaffer, O.C., Bard, J.A.M., Husain, K., Kik, S.K., et al., (2023). Adaptive preservation of orphan ribosomal proteins in chaperone-dispersed condensates. *Nature Cell Biol.* **25**, 1691–1703.
129. Yamanaka, T., Miyazaki, H., Oyama, F., Kurosawa, M., Washizu, C., Doi, H., Nukina, N., (2008). Mutant Huntingtin reduces HSP70 expression through the sequestration of NF-Y transcription factor. *EMBO J.* **27**, 827–839.
130. Chafekar, S.M., Duennwald, M.L., (2012). Impaired heat shock response in cells expressing full-length polyglutamine-expanded huntingtin. *PLoS One* **7**, e37929.
131. Deriziotis, P., Andre, R., Smith, D.M., Goold, R., Kinghorn, K.J., Kristiansen, M., et al., (2011). Misfolded PrP impairs the UPS by interaction with the 20S proteasome and inhibition of substrate entry. *EMBO J.* **30**, 3065–3077.
132. Hayes, L.R., Duan, L., Bowen, K., Kalab, P., Rothstein, J. D., (2020). C9orf72 arginine-rich dipeptide repeat proteins disrupt karyopherin-mediated nuclear import. *Elife* **9**
133. Anckar, J., Sistonen, L., (2011). Regulation of HSF1 function in the heat stress response: implications in aging and disease. *Annu. Rev. Biochem* **80**, 1089–1115.
134. Roth, D.M., Hutt, D.M., Tong, J., Bouchecareilh, M., Wang, N., Seeley, T., et al., (2014). Modulation of the maladaptive stress response to manage diseases of protein folding. *PLoS Biol.* **12**, e1001998.
135. Klaips, C.L., Gropp, M.H.M., Hipp, M.S., Hartl, F.U., (2020). Sis1 potentiates the stress response to protein aggregation and elevated temperature. *Nature Commun.* **11**, 6271.
136. Gaglia, G., Rashid, R., Yapp, C., Joshi, G.N., Li, C.G., Lindquist, S.L., et al., (2020). HSF1 phase transition mediates stress adaptation and cell fate decisions. *Nature Cell Biol.* **22**, 151–158.
137. Westerheide, S.D., Anckar, J., Stevens Jr., S.M., Sistonen, L., Morimoto, R.I., (2009). Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science* **323**, 1063–1066.
138. Raychaudhuri, S., Loew, C., Korner, R., Pinkert, S., Theis, M., Hayer-Hartl, M., et al., (2014). Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1. *Cell* **156**, 975–985.
139. Bennett, E.J., Bence, N.F., Jayakumar, R., Kopito, R.R., (2005). Global impairment of the ubiquitin-proteasome system by nuclear or cytoplasmic protein aggregates precedes inclusion body formation. *Mol. Cell* **17**, 351–365.
140. Kristiansen, M., Deriziotis, P., Dimcheff, D.E., Jackson, G. S., Ovaa, H., Naumann, H., et al., (2007). Disease-associated prion protein oligomers inhibit the 26S proteasome. *Mol. Cell* **26**, 175–188.
141. Urushitani, M., Kurisu, J., Tsukita, K., Takahashi, R., (2002). Proteasomal inhibition by misfolded mutant superoxide dismutase 1 induces selective motor neuron death in familial amyotrophic lateral sclerosis. *J. Neurochem.* **83**, 1030–1042.
142. Riemenschneider, H., Guo, Q., Bader, J., Frottin, F., Farny, D., Kleinberger, G., et al., (2022). Gel-like inclusions of C-terminal fragments of TDP-43 sequester stalled proteasomes in neurons. *EMBO Rep.* **23**, e53890.
143. Juenemann, K., Schipper-Krom, S., Wiemhoefer, A., Kloss, A., Sanz Sanz, A., Reits, E.A.J., (2013). Expanded polyglutamine-containing N-terminal huntingtin fragments are entirely degraded by mammalian proteasomes. *J. Biol. Chem.* **288**, 27068–27084.
144. Rousseau, E., Kojima, R., Hoffner, G., Djian, P., Bertolotti, A., (2009). Misfolding of proteins with a polyglutamine expansion is facilitated by proteasomal chaperones. *J. Biol. Chem.* **284**, 1917–1929.
145. Blumenstock, S., Schulz-Trieglaff, E.K., Voelkl, K., Bolender, A.L., Lapios, P., Lindner, J., et al., (2021). Fluc-EGFP reporter mice reveal differential alterations of neuronal proteostasis in aging and disease. *EMBO J.* e107260.
146. Sui, X., Pires, D.E.V., Ormsby, A.R., Cox, D., Nie, S., Vecchi, G., et al., (2020). Widespread remodeling of proteome solubility in response to different protein homeostasis stresses. *PNAS* **117**, 2422–2431.
147. Wang, J., Farr, G.W., Zeiss, C.J., Rodriguez-Gil, D.J., Wilson, J.H., Furtak, K., et al., (2009). Progressive aggregation despite chaperone associations of a mutant SOD1-YFP in transgenic mice that develop ALS. *PNAS* **106**, 1392–1397.
148. Liu, F., Morderer, D., Wren, M.C., Vettleson-Trutza, S.A., Wang, Y., Rabichow, B.E., et al., (2022). Proximity proteomics of C9orf72 dipeptide repeat proteins identifies molecular chaperones as modifiers of poly-GA aggregation. *Acta Neuropathol. Commun.* **10**, 22.
149. Panda, P., Sarohi, V., Basak, T., Kasturi, P., (2023). Elucidation of site-specific ubiquitination on chaperones in response to mutant huntingtin. *Cell. Mol. Neurobiol.* **44**, 3.
150. Deng, H.X., Chen, W., Hong, S.T., Boycott, K.M., Gorrie, G.H., Siddique, N., et al., (2011). Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature* **477**, 211–215.
151. Fecto, F., Yan, J., Vemula, S.P., Liu, E., Yang, Y., Chen, W., et al., (2011). SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. *Arch. Neurol.* **68**, 1440–1446.
152. Lu, S., Hu, J., Arogundade, O.A., Goginashvili, A., Vazquez-Sanchez, S., Diedrich, J.K., et al., (2022). Heat-shock chaperone HSPB1 regulates cytoplasmic TDP-43 phase separation and liquid-to-gel transition. *Nature Cell Biol.* **24**, 1378–1393.
153. Capponi, S., Geuens, T., Geroldi, A., Origone, P., Verdiani, S., Cichero, E., et al., (2016). Molecular chaperones in the pathogenesis of amyotrophic lateral sclerosis: the role of HSPB1. *Hum. Mutat.* **37**, 1202–1208.
154. Woehlbier, U., Colombo, A., Saaranen, M.J., Perez, V., Ojeda, J., Bustos, F.J., et al., (2016). ALS-linked protein disulfide isomerase variants cause motor dysfunction. *EMBO J.* **35**, 845–865.
155. Johnson, J.O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V.M., Trojanowski, J.Q., et al., (2010). Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* **68**, 857–864.
156. DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., et al., (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72**, 245–256.

157. Renton, A.E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J.R., et al., (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* **72**, 257–268.
158. Balendra, R., Isaacs, A.M., (2018). C9orf72-mediated ALS and FTD: multiple pathways to disease. *Nature Rev. Neurol.* **14**, 544–558.
159. Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., et al., (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**, 605–608.
160. Valente, E.M., Abou-Sleiman, P.M., Caputo, V., Muqit, M. M., Harvey, K., Gispert, S., et al., (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304**, 1158–1160.
161. Bader, V., Winklhofer, K.F., (2020). PINK1 and Parkin: team players in stress-induced mitophagy. *Biol. Chem.* **401**, 891–899.
162. Lambert, J.C., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M., et al., (2009). Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nature Genet.* **41**, 1094–1099.
163. Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M.L., et al., (2009). Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nature Genet.* **41**, 1088–1093.
164. Yuste-Checa, P., Trinkaus, V.A., Riera-Tur, I., Imamoglu, R., Schaller, T.F., Wang, H., et al., (2021). The extracellular chaperone Clusterin enhances Tau aggregate seeding in a cellular model. *Nature Commun.* **12**, 4863.
165. Yuste-Checa, P., Bracher, A., Hartl, F.U., (2022). The chaperone Clusterin in neurodegeneration-friend or foe? *Bioessays* **44**, e2100287.
166. Anttonen, A.K., Mahjneh, I., Hamalainen, R.H., Lagier-Tourenne, C., Kopra, O., Waris, L., et al., (2005). The gene disrupted in Marinesco-Sjogren syndrome encodes SIL1, an HSPA5 cochaperone. *Nature Genet.* **37**, 1309–1311.
167. Senderek, J., Krieger, M., Stendel, C., Bergmann, C., Moser, M., Breitbach-Faller, N., et al., (2005). Mutations in SIL1 cause Marinesco-Sjogren syndrome, a cerebellar ataxia with cataract and myopathy. *Nature Genet.* **37**, 1312–1314.
168. Hansen, J.J., Durr, A., Cournu-Rebeix, I., Georgopoulos, C., Ang, D., Nielsen, M.N., et al., (2002). Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60. *Am. J. Hum. Genet.* **70**, 1328–1332.
169. Magen, D., Georgopoulos, C., Bross, P., Ang, D., Segev, Y., Goldsher, D., et al., (2008). Mitochondrial hsp60 chaperonopathy causes an autosomal-recessive neurodegenerative disorder linked to brain hypomyelination and leukodystrophy. *Am. J. Hum. Genet.* **83**, 30–42.
170. Chiti, F., Kelly, J.W., (2022). Small molecule protein binding to correct cellular folding or stabilize the native state against misfolding and aggregation. *Curr. Opin. Struct. Biol.* **72**, 267–278.
171. Bulawa, C.E., Connelly, S., Devit, M., Wang, L., Weigel, C., Fleming, J.A., et al., (2012). Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. *PNAS* **109**, 9629–9634.
172. Mijnders, M., Kleizen, B., Braakman, I., (2017). Correcting CFTR folding defects by small-molecule correctors to cure cystic fibrosis. *Curr. Opin. Pharmacol.* **34**, 83–90.
173. Fiedorczuk, K., Chen, J., (2022). Mechanism of CFTR correction by type I folding correctors. *Cell* **185**, 158–168. e11.
174. Sittler, A., Lurz, R., Lueder, G., Priller, J., Lehrach, H., Hayer-Hartl, M.K., et al., (2001). Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum. Mol. Genet.* **10**, 1307–1315.
175. Auluck, P.K., Chan, H.Y., Trojanowski, J.Q., Lee, V.M., Bonini, N.M., (2002). Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* **295**, 865–868.
176. Schaffar, G., Breuer, P., Boteva, R., Behrends, C., Tzvetkov, N., Strippel, N., et al., (2004). Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. *Mol. Cell* **15**, 95–105.
177. Bersuker, K., Hipp, M.S., Calamini, B., Morimoto, R.I., Kopito, R.R., (2013). Heat shock response activation exacerbates inclusion body formation in a cellular model of Huntington disease. *J. Biol. Chem.* **288**, 23633–23638.
178. Tsaytler, P., Harding, H.P., Ron, D., Bertolotti, A., (2011). Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science* **332**, 91–94.
179. Das, I., Krzyzosiak, A., Schneider, K., Wrabetz, L., D'Antonio, M., Barry, N., et al., (2015). Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science* **348**, 239–242.
180. Djajadikerta, A., Keshri, S., Pavel, M., Prestil, R., Ryan, L., Rubinsztein, D.C., (2020). Autophagy Induction as a Therapeutic Strategy for Neurodegenerative Diseases. *J. Mol. Biol.* **432**, 2799–2821.
181. Lokireddy, S., Kukushkin, N.V., Goldberg, A.L., (2015). cAMP-induced phosphorylation of 26S proteasomes on Rpn6/PSMD11 enhances their activity and the degradation of misfolded proteins. *PNAS* **112**, E7176–E7185.
182. Khosravi, B., LaClair, K.D., Riemenschneider, H., Zhou, Q., Frottin, F., Mareljic, N., et al., (2020). Cell-to-cell transmission of C9orf72 poly-(Gly-Ala) triggers key features of ALS/FTD. *EMBO J.* **39**, e102811.
183. Lee, B.H., Lee, M.J., Park, S., Oh, D.C., Elsasser, S., Chen, P.C., et al., (2010). Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* **467**, 179–184.
184. Guay, K.P., Ke, H., Canniff, N.P., George, G.T., Eyles, S. J., Mariappan, M., et al., (2023). ER chaperones use a protein folding and quality control glyco-code. *Mol. Cell* **83**, 4524–4537.e5.
185. Gidalevitz, T., Prahlad, V., Morimoto, R.I., (2011). The stress of protein misfolding: from single cells to multicellular organisms. *Cold Spring Harb. Perspect. Biol.* **3**
186. Sidrauski, C., Acosta-Alvear, D., Khoutorsky, A., Vedantham, P., Hearn, B.R., Li, H., et al., (2013). Pharmacological brake-release of mRNA translation enhances cognitive memory. *Elife* **2**, e00498.

187. Leitman, J., Barak, B., Benyair, R., Shenkman, M., Ashery, U., Hartl, F.U., Lederkremer, G.Z., (2014). ER stress-induced eIF2-alpha phosphorylation underlies sensitivity of striatal neurons to pathogenic huntingtin. *PLoS One* **9**, e90803.
188. Lv, X., Lu, X., Cao, J., Luo, Q., Ding, Y., Peng, F., et al., (2023). Modulation of the proteostasis network promotes tumor resistance to oncogenic KRAS inhibitors. *Science* **381**, eabn4180
189. Scherz-Shouval, R., Santagata, S., Mendillo, M.L., Sholl, L.M., Ben-Aharon, I., Beck, A.H., et al., (2014). The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. *Cell* **158**, 564–578.
190. Mendillo, M.L., Santagata, S., Koeva, M., Bell, G.W., Hu, R., Tamimi, R.M., et al., (2012). HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. *Cell* **150**, 549–562.
191. Doyle, S.M., Genest, O., Wickner, S., (2013). Protein rescue from aggregates by powerful molecular chaperone machines. *Nature Rev. Mol. Cell Biol.* **14**, 617–629.
192. Nillegoda, N.B., Wentink, A.S., Bukau, B., (2018). Protein Disaggregation in Multicellular Organisms. *Trends Biochem. Sci* **43**, 285–300.
193. Faust, O., Abayev-Avraham, M., Wentink, A.S., Maurer, M., Nillegoda, N.B., London, N., et al., (2020). HSP40 proteins use class-specific regulation to drive HSP70 functional diversity. *Nature*.
194. Wentink, A.S., Nillegoda, N.B., Feufel, J., Ubartaite, G., Schneider, C.P., De Los, R.P., et al., (2020). Molecular dissection of amyloid disaggregation by human HSP70. *Nature*.
195. Shorter, J., (2011). The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLoS One* **6**, e26319.
196. Nachman, E., Wentink, A.S., Madiona, K., Bousset, L., Katsinelos, T., Allinson, K., et al., (2020). Disassembly of Tau fibrils by the human Hsp70 disaggregation machinery generates small seeding-competent species. *J. Biol. Chem.*.
197. Schneider, M.M., Gautam, S., Herling, T.W., Andrzejewska, E., Krainer, G., Miller, A.M., et al., (2021). The Hsc70 disaggregation machinery removes monomer units directly from alpha-synuclein fibril ends. *Nature Commun.* **12**, 5999.
198. Gao, X., Carroni, M., Nussbaum-Krammer, C., Mogk, A., Nillegoda, N.B., Szlachcic, A., et al., (2015). Human Hsp70 disaggregase reverses Parkinson's-linked alpha-synuclein amyloid fibrils. *Mol. Cell* **59**, 781–793.
199. Scior, A., Buntru, A., Arnsburg, K., Ast, A., Iburg, M., Juenemann, K., et al., (2018). Complete suppression of Htt fibrilization and disaggregation of Htt fibrils by a trimeric chaperone complex. *EMBO J.* **37**, 282–299.
200. Darwich, N.F., Phan, J.M., Kim, B., Suh, E., Papatriantafyllou, J.D., Changolkar, L., et al., (2020). Autosomal dominant VCP hypomorph mutation impairs disaggregation of PHF-tau. *Science* **370**
201. Sontag, E.M., Samant, R.S., Frydman, J., (2017). Mechanisms and functions of spatial protein quality control. *Annu. Rev. Biochem* **86**, 97–122.
202. Cliffe, R., Sang, J.C., Kundel, F., Finley, D., Klenerman, D., Ye, Y., (2019). Filamentous aggregates are fragmented by the proteasome holoenzyme. *Cell Rep.* **26**, 2140–2149e3.
203. Zhu, G., Harischandra, D.S., Ghaisas, S., Zhang, P., Prall, W., Huang, L., et al., (2020). TRIM11 prevents and reverses protein aggregation and rescues a mouse model of parkinson's disease. *Cell Rep.* **33**, 108418
204. Tittelmeier, J., Sandhof, C.A., Ries, H.M., Druffel-Augustin, S., Mogk, A., Bukau, B., Nussbaum-Krammer, C., (2020). The HSP110/HSP70 disaggregation system generates spreading-competent toxic alpha-synuclein species. *EMBO J.* **39**, e103954.
205. Jackrel, M.E., DeSantis, M.E., Martinez, B.A., Castellano, L.M., Stewart, R.M., Caldwell, K.A., et al., (2014). Potentiated Hsp104 variants antagonize diverse proteotoxic misfolding events. *Cell* **156**, 170–182.
206. Shorter, J., (2016). Engineering therapeutic protein disaggregases. *Mol. Biol. Cell* **27**, 1556–1560.
207. Mack, K.L., Kim, H., Barbieri, E.M., Lin, J., Braganza, S., Jackrel, M.E., et al., (2023). Tuning Hsp104 specificity to selectively detoxify alpha-synuclein. *Mol. Cell*.
208. Franzmann, T.M., Jahnel, M., Pozniakovsky, A., Mahamid, J., Holehouse, A.S., Nuske, E., et al., (2018). Phase separation of a yeast prion protein promotes cellular fitness. *Science* **359**
209. Mateju, D., Franzmann, T.M., Patel, A., Kopach, A., Boczek, E.E., Maharana, S., et al., (2017). An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. *EMBO J.* **36**, 1669–1687.
210. Shin, Y., Brangwynne, C.P., (2017). Liquid phase condensation in cell physiology and disease. *Science* **357**
211. Musacchio, A., (2022). On the role of phase separation in the biogenesis of membraneless compartments. *EMBO J.* **41**, e109952.
212. Banani, S.F., Lee, H.O., Hyman, A.A., Rosen, M.K., (2017). Biomolecular condensates: organizers of cellular biochemistry. *Nature Rev. Mol. Cell Biol.* **18**, 285–298.
213. Riback, J.A., Katanski, C.D., Kear-Scott, J.L., Pilipenko, E.V., Rojek, A.E., Sosnick, T.R., Drummond, D.A., (2017). Stress-triggered phase separation is an adaptive, evolutionarily tuned response. *Cell* **168**, 1028–1040e19.
214. Nakashima, K.K., Vibhute, M.A., Spruijt, E., (2019). Biomolecular chemistry in liquid phase separated compartments. *Front. Mol. Biosci.* **6**, 21.
215. Alberti, S., Hyman, A.A., (2021). Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. *Nature Rev. Mol. Cell Biol.* **22**, 196–213.
216. Patel, A., Lee, H.O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M.Y., et al., (2015). A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* **162**, 1066–1077.
217. Gopal, P.P., Nirschl, J.J., Klinman, E., Holzbaur, E.L., (2017). Amyotrophic lateral sclerosis-linked mutations increase the viscosity of liquid-like TDP-43 RNP granules in neurons. *PNAS* **114**, E2466–E2475.
218. Mediani, L., Guillen-Boixet, J., Vinet, J., Franzmann, T.M., Bigi, I., Mateju, D., et al., (2019). Defective ribosomal products challenge nuclear function by impairing nuclear condensate dynamics and immobilizing ubiquitin. *EMBO J.* **38**, e101341.
219. Frottin, F., Perez-Berlanga, M., Hartl, F.U., Hipp, M.S., (2021). Multiple pathways of toxicity induced by C9orf72

dipeptide repeat aggregates and G4C2 RNA in a cellular model. *Elife*, 10.

220. Kwon, I., Xiang, S., Kato, M., Wu, L., Theodoropoulos, P., Wang, T., et al., (2014). Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. *Science* **345**, 1139–1145.
221. Lee, K.H., Zhang, P., Kim, H.J., Mitrea, D.M., Sarkar, M., Freibaum, B.D., et al., (2016). C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles. *Cell* **167**, 774–788e17.
222. Boyko, S., Surewicz, W.K., (2022). Tau liquid-liquid phase separation in neurodegenerative diseases. *Trends Cell Biol.* **32**, 611–623.
223. Ray, S., Singh, N., Kumar, R., Patel, K., Pandey, S., Datta, D., et al., (2020). alpha-Synuclein aggregation nucleates through liquid-liquid phase separation. *Nature Chem.* **12**, 705–716.
224. Peskett, T.R., Rau, F., O'Driscoll, J., Patani, R., Lowe, A. R., Saibil, H.R., (2018). A Liquid to solid phase transition underlying pathological huntingtin Exon1 aggregation. *Mol. Cell* **70**, 588–601e6.
225. Liu, Z., Zhang, S., Gu, J., Tong, Y., Li, Y., Gui, X., et al., (2020). Hsp27 chaperones FUS phase separation under the modulation of stress-induced phosphorylation. *Nature Struct. Mol. Biol.* **27**, 363–372.
226. Yu, H., Lu, S., Gasior, K., Singh, D., Vazquez-Sanchez, S., Tapia, O., et al., (2021). HSP70 chaperones RNA-free TDP-43 into anisotropic intranuclear liquid spherical shells. *Science* **371**
227. Li, Y., Gu, J., Wang, C., Hu, J., Zhang, S., Liu, C., et al., (2022). Hsp70 exhibits a liquid-liquid phase separation ability and chaperones condensed FUS against amyloid aggregation. *iScience* **25**, 104356
228. Alberti, S., Hyman, A.A., (2016). Are aberrant phase transitions a driver of cellular aging? *Bioessays* **38**, 959–968.
229. Zhao DY, Bäuerlein FJB, Saha I, Hartl FU, Baumeister W, Wilfling F.Mol Cell. Autophagy preferentially degrades non-fibrillar polyQ aggregates. 2024 May 16;84(10):1980-1994.e8. doi: 10.1016/j.molcel.2024.04.018. PMID: 38759629.