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*Published in:*
Journal of Molecular Biology

*DOI:*
10.1016/j.jmb.2024.168615

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*
Publisher's PDF, also known as Version of record

*Publication date:*
2024

Link to publication in University of Groningen/UMCG research database

*Citation for published version (APA):*
Hipp, M. S., & Hartl, F. U. (2024). Interplay of Proteostasis Capacity and Protein Aggregation: Implications for Cellular Function and Disease. *Journal of Molecular Biology*, *436*(14), Article 168615. https://doi.org/10.1016/j.jmb.2024.168615

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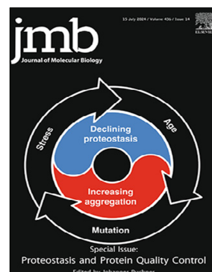


# Interplay of Proteostasis Capacity and Protein Aggregation: Implications for Cellular Function and Disease

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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.
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## 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

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]

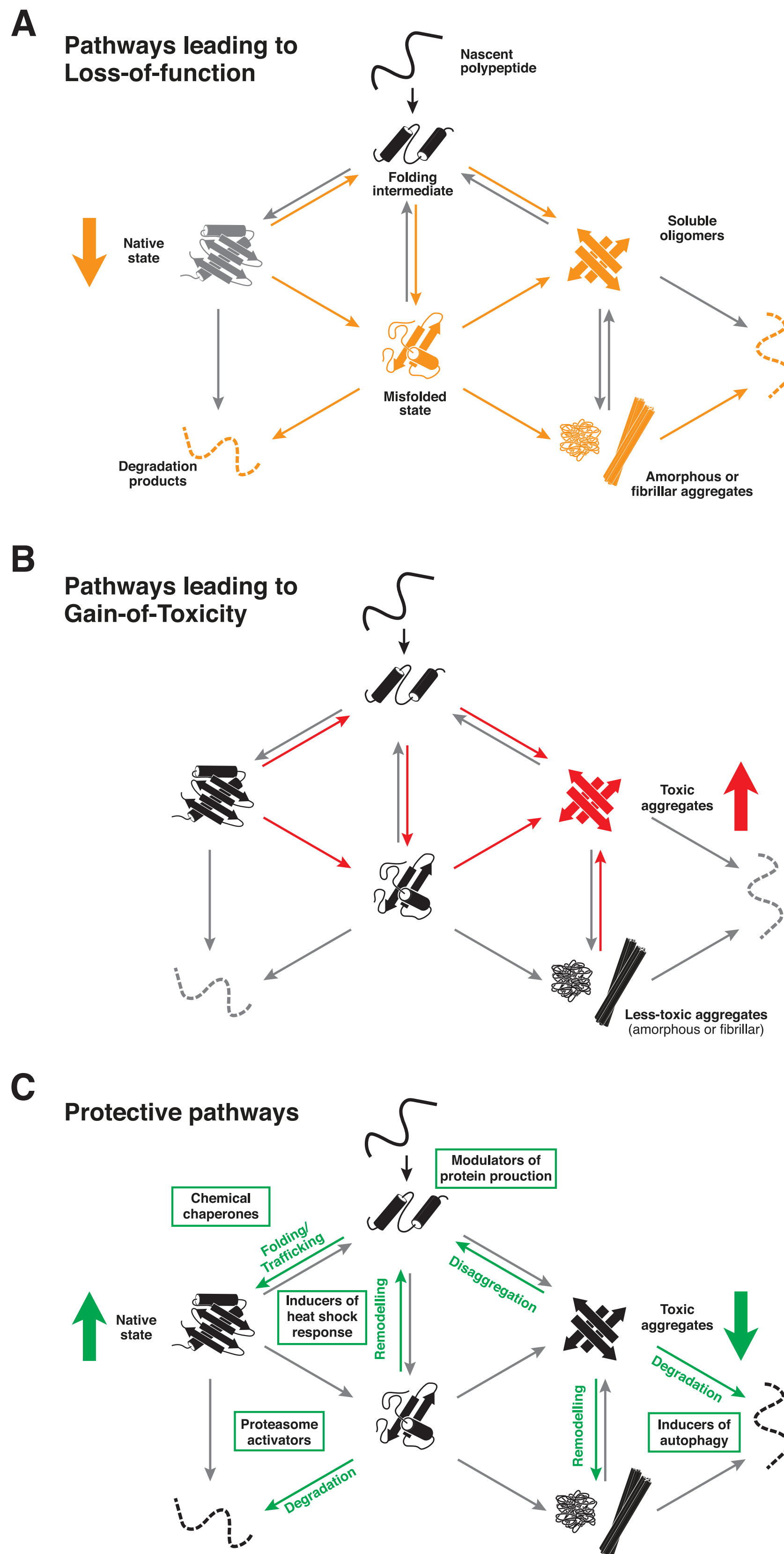
Notably, some phase separated compartments may undergo a transition from liquid-like to more solid structures, characterized by increased molecular packing and reduced fluidity.[215] This transition is influenced by various factors, including molecular concentration, post-translational modifications, 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
Remodelling
Inducers of autophagy
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-

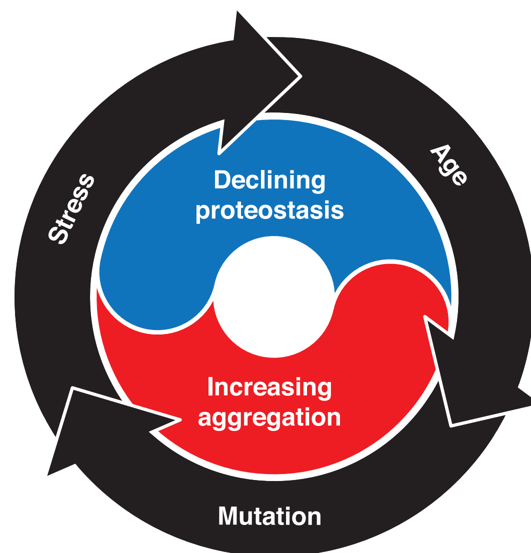



**Figure 2. Feed-forward loop between proteostasis decline and increased aggregation.** A reduced capacity of the PN increases the risk of protein aggregation. On the other hand, protein aggregates titrate PN components, and reduce the available proteostasis capacity. Both aspects may initiate a feed-forward 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 16 May 2024*

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

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