Proteostasis impairment in protein misfolding and aggregation diseases

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Abstract

Cells possess an extensive network of components to safeguard proteome integrity and maintain protein homeostasis (proteostasis). When this proteostasis network (PN) declines in performance, as may be the case during aging, newly-synthesized proteins are no longer able to fold efficiently and metastable proteins lose their functionally active conformations, particularly under conditions of cell stress. Apart from loss-of-function effects, a critical consequence of PN deficiency is the accumulation of cytotoxic protein aggregates, which are also associated with many age-dependent neurodegenerative diseases and other medical disorders. Here we discuss recent evidence that the chronic production of aberrantly folded and aggregated proteins in these diseases is harmful by overtaxing PN capacity, setting in motion a vicious cycle of increasing proteome imbalance that eventually leads to PN collapse and cell death.

Keywords:

Proteostasis, protein aggregation, protein (mis)folding, degradation, molecular chaperones, neurodegenerative disease

Aggregates and proteostasis

The bulk of cellular functions are carried out by proteins. To maintain protein homeostasis (or proteostasis [1]) – the state of proteome balance – mammalian cells must ensure that more than 10,000 different proteins fold and assemble efficiently upon synthesis and preserve their functionally active states in a wide range of environmental and metabolic conditions. This is a challenging task, since proteins are only marginally stable at physiological temperature and are constantly at risk of misfolding. In addition, the concentration and subcellular localization of each individual protein species needs to be carefully controlled. Proteostasis is maintained by a plethora of factors, including molecular chaperones and their regulators as well as the machineries of proteolytic degradation (~1400 proteins in mammalian cells [2, 3]). An emerging concept of recent research is that these components function as a coordinated proteostasis network (PN). The organizational principle of this network is far from being understood but it is clear that the PN has evolved to ensure proteome integrity and prevent the accumulation of aberrant conformational states, specifically protein aggregates, which are increasingly recognized as the cause of cytotoxicity in neurodegenerative diseases and other medical disorders, ranging from Alzheimer's disease (AD) to type 2 diabetes [4].

We define a protein aggregate as any association of two or more protein molecules in a non-native conformation. Aggregates cover a range of structures, from amorphous assemblies to highly ordered fibrils (amyloid) with cross-β-structure. The propensity of a specific protein to aggregate is governed primarily by the chemical properties of its amino acid sequence, the conformational stability of its folded state and its cellular concentration [5, 6]. The extremely high total protein concentration in

cells (~300 g/L) results in excluded volume effects (macromolecular crowding) and substantially increases the tendency of non-native protein molecules to aggregate compared to dilute solutions [7]. Moreover, ~30% of proteins in higher eukaryotes contain extensive intrinsically unstructured regions (>30 amino acids in length) [8]. These proteins are often metastable and some of them are particularly toxic when they aggregate, like α-synuclein in Parkinson's disease (PD) and Aβ and Tau in AD. The presence of aggregates is indicative of proteostasis imbalance. However, aggregate formation is not only the *result* of insufficient proteostasis capacity, but can also be the *cause* of PN imbalance by overburdening available chaperone and degradation machineries, thereby enforcing a self-propagating cycle that eventually leads to proteostasis collapse and cell death (Figure 1).

In this review we summarize recent research addressing the interrelationship between protein aggregation and the functional status of the PN. We propose that the failure of cells to maintain proteostasis contributes to the toxic effects of protein aggregates in numerous diseases and is also a major driver of the aging process. Correcting PN imbalance pharmacologically [2] presents an opportunity for the development of novel therapeutic strategies.

Components of the PN

While it has long been known that a large fraction of proteins require chaperone assistance for initial folding, we are only beginning to realize the extent to which proteins rely on chaperone-dependent functions throughout their cellular lifetime in order to maintain or regain their biologically active conformations [1, 3]. Core activities of the chaperone machinery include aggregation prevention (so-called 'holdase' function), the ability to refold aberrantly folded states, and also the capacity

to actively dissociate certain protein aggregates (Figure 2). The archetypical holdase chaperones are the so-called small heat shock proteins (sHSPs), which buffer aggregation by binding non-native protein species via hydrophobic interactions [9]. They cooperate with Hsp70 chaperones, which refold proteins through ATP-dependent cycles of protein binding and release and are subject to regulation by multiple co-chaperones (Hsp40s and nucleotide exchange factors) [10]. The Hsp70s also participate in protein disaggregation, sometimes in cooperation with AAA ATPase-chaperones, such as Hsp104 in fungi [11]. The Hsp70 system also functions with Hsp90 and its multiple co-factors in folding and regulating many conformationally dynamic proteins, including kinases and other signaling molecules [12].

Protein synthesis and turnover must be carefully balanced and terminally misfolded proteins effectively removed by proteolysis to ensure proteostasis. Clearance of misfolded proteins is mainly performed by the ubiquitin-proteasome system (UPS) (Figure 2), comprising more than 600 proteins in human cells [13, 14]. Proteasome complexes are localized in the cytosol and nucleus. Proteins of the ER destined for degradation must first undergo retrotranslocation to the cytosol to gain access to the proteasome [15, 16]. Specific factors of the UPS cooperate closely with chaperone machinery. For example, the co-chaperone and ubiquitin ligase CHIP binds to Hsp70 and Hsp90, mediating ubiquitylation of faulty client proteins and guiding them to the proteasome [17]. Proteins that are unable to fold – due to mutation or amino acid misincorporation, or as a result of oxidative damage – will spend more time in the chaperone-bound state, thus increasing the chance of being recognized by CHIP and other ubiquitin ligases. Importantly, proteins need to be unfolded prior to proteasomal degradation in a reaction that is mediated by the AAA ATPase

components of the proteasome complex [18]. For this mechanism to be efficient, proteins must be delivered in a soluble, non-aggregated state and incipient aggregates [19, 20] may be actively dissociated by chaperone machinery to allow degradation via the UPS.

Larger protein aggregates and insoluble inclusions, resistant to dissociation, can be removed by autophagy and lysosomal degradation, the other major pathway of proteolysis (Figure 2). Autophagy involves the engulfment of material by a double membrane vesicle (the autophagosome) that subsequently fuses with the lysosome [21]. Unlike the non-specific autophagy of bulk cytoplasm, aggregates are subject to selective autophagy. This process is assisted by the Hsp70 chaperone system, involving ubiquitylation of target proteins by CHIP and recruitment of the autophagic ubiquitin adaptor p62 by the Hsp70 co-factor Bag-3 [22]. Alternatively, Bag-3 may mediate selective autophagy independent of substrate ubiquitylation [23]. There is also evidence that protein aggregates are first actively concentrated in aggresomes [24] or juxtanuclear quality control compartments (JUNQs) [25] by cytoskeleton-based transport processes [26, 27], followed by recruitment of autophagic machinery [28]. Aggresome and JUNQ formation are thought to allow the deposition of aggregated proteins in a non-toxic storage form until sufficient capacity for degradation is available [29]. Some misfolded protein species are delivered directly to lysosomes by cytosolic Hsp70 in a manner involving recognition of a specific peptide motif, KFERQ, present in many proteins [30].

Age-dependent proteostasis decline

Age is the major risk factor for numerous aggregate deposition diseases, including AD, PD, Huntington's disease (HD) and other degenerative disorders. Studies in

lower invertebrates such as the nematode *C. elegans* suggest that this is due to a decline in the capacity of aging cells and tissues to maintain proteostasis and to respond adequately to protein conformational stresses by upregulating PN machinery [31-33]. Whether an active aging program or simply lack of evolutionary pressure underlies this deterioration is unclear. It has been argued that organisms gained evolutionary advantage by devoting more resources to propagating the germ line than to maintaining the integrity of the somatic proteome ('disposable soma theory' [34]). Indeed, in *C. elegans* proteostasis deteriorates dramatically soon after progeny have been produced [33]. It has also been suggested that pluripotent stem cells dedicate considerably more resources to proteome maintenance than differentiated cells [35]. Although in mammals the age-dependent proteostasis decline is probably more protracted [36] than in a short-lived metazoan like *C. elegans*, the accumulation of aberrant protein species eventually exhausts the capacity of the PN and results in cellular dysfunction [32, 37]. Not surprisingly, this problem manifests itself most severely in post-mitotic cells (neurons and muscle cells) which lack the ability to remove aggregates by retaining them in the mother cell during cell division [38, 39].

Aggregate deposition diseases, including the major age-dependent neurodegenerative disorders, are typically associated with a gain-of-toxic-function, in contrast to loss-of-function diseases like cystic fibrosis [1], in which specific proteins – unable to fold due to mutation – are removed by degradation. While a connection between proteostasis decline and aggregation can be observed in most of the major degenerative diseases [40, 41], the analysis of pathological polyQ proteins in various model systems has provided the best evidence to date that age-dependent proteostasis decline is a prerequisite for disease manifestation and that protein aggregation in turn causes proteostasis impairment [42-45] (Figure 1). PolyQ-expansion diseases,

including HD) (Box 1), are inherited in a dominant manner and no sporadic cases are known, unlike most other aggregate deposition disorders. In contrast to the familiar forms of AD, PD and amyotrophic lateral sclerosis (ALS), HD is caused by mutation of a single gene. Moreover, the clear correlation between polyQ repeat length and aggregation propensity and the inverse correlation between repeat length and the age of disease onset greatly facilitates the development of disease models.

Cellular dysfunction and cell death in neurodegenerative disease appears to be mainly caused by a subset of highly toxic aggregate species, including diffusible, oligomeric forms that lack ordered fibrillar topology [6] (Figure 2A). These toxic species are structurally ill-defined but are thought to expose hydrophobic amino acid residues on unpaired β-strands that provide 'sticky' surfaces for aberrant interactions with other proteins or cellular membranes [6, 46, 47]. Multiple endogenous proteins, often newly-synthesized or containing extensive disordered regions, as well as certain chaperones have been found associated with such aggregates [48]. Importantly, there is increasing evidence that sequestration of oligomers into large insoluble deposits is protective [49], presumably by reducing the interactive, solvent exposed surface area of the aggregates. However, while inclusion formation may be actively promoted by the PN of aged cells as a last resort [42], these deposits are unlikely to be entirely harmless.

Mechanisms of proteostasis impairment

Observations in cellular and organismal models indicate that the chronic production of misfolded and aggregated proteins compromises central functions of the PN, including the capacity of cells to fold proteins, clear misfolded proteins (Figure 3A,B) and to respond to conformational stress by upregulating PN machinery (Figure 3C)

[43-45]. How exactly the aberrant proteins target the PN is not clear in most cases, but it is plausible that they unduly occupy, sequester or otherwise functionally impair PN components, rendering them unavailable for use by other clients (chaperone titration). These clients comprise primarily the 'metastable proteome', a set of structurally dynamic proteins that need constant chaperone surveillance. Importantly, the acute accumulation of misfolded proteins under stress conditions (such a heat stress) causes the rapid induction of PN components to reestablish proteome balance. However, this fails to occur when aberrant protein species are produced chronically, as in disease or during aging [48, 50-52] (Figure 3C). One possible explanation is that under chronic stress, key components of stress signaling pathways can no longer be maintained in a functionally active state. For example, NF-Y, a transcription factor involved in Hsp70 expression is sequestered by polyQ aggregates [53], and heat-shock transcription factor 1 (HSF-1), the key regulator of the cytosolic stress response, is itself a metastable protein whose level is regulated by chaperones and proteasomal degradation [54, 55].

Much research in recent years has focused on understanding how protein aggregation interferes with the function of the UPS. Ubiquitin is present in the inclusions of nearly all neurodegenerative disease proteins, consistent with the view that these proteins fail to be degraded when they accumulate to levels that exceed proteasome capacity [56, 57]. A decline in proteasome activity during aging [58, 59] would explain why aging is a key risk factor for protein aggregation. Conversely, there is evidence that aggregation is not the result of a malfunctioning UPS but is actually its cause. This view is supported by findings that the expression of structurally unrelated aggregation-prone proteins prevents other proteins from being proteasomally degraded. Examples include polyQ-expansion proteins, the disease-

associated prion protein Prp^{Sc}, mutants of superoxide dismutase 1 (SOD1) linked with ALS and mutants of rhodopsin linked to autosomal dominant retinitis pigmentosa [60-64]. Evidence has been presented that the aggregates engage the proteasome but cannot be unfolded, thus 'choking' the system [65] and interfering with the entry of other substrates [66]. However, polyQ aggregates do not inhibit proteasome function in vitro [61, 67] and polyQ proteins are efficiently degraded in yeast and mammalian cells when they are targeted to the proteasome by N- or C terminal degradation signals [44, 68].

How then does protein aggregation compromise the UPS? Support for a mechanism in which the aggregates sequester and functionally deplete critical components of the PN was recently provided using a yeast model (Figure 3). It was shown that expression of polyQ-expanded huntingtin exon 1 fragment causes the stabilization of terminally misfolded proteins that normally undergo rapid degradation via the UPS [44]. This effect was mediated by binding of the Hsp40 chaperone Sis1 to soluble polyQ oligomers and its sequestration into insoluble inclusions. Sis1 is an essential but low abundant regulator of the Hsp70 chaperone system, and homologs of Sis1 are potent modulators of polyQ aggregation in mammalian cells when overexpressed [44, 69]. Surprisingly, Sis1 was found to be rate-limiting for the transport of several cytosolic misfolded proteins into the nucleus (Box 2), where they are degraded by nuclear proteasomes [44, 70, 71]. Even when Sis1 was only mildly depleted by the polyQ aggregates, misfolded proteins aggregated in the cytosol, indicating that Sis1 has a critical function in the PN [44]. Interestingly, the human Sis1 homolog, DnaJB1, was found in polyQ inclusions of postmortem brain tissue from spinocerebellar ataxia type 3 patients [72], suggesting that proteostasis impairment resulting from Hsp40 sequestration is relevant in disease. Chaperone

sequestration by aggregation-prone proteins is likely to play a role in various neurodegenerative diseases. For example, the aggregates of mutant SOD1, associated with ALS, sequester Hsc70 and its nucleotide exchange factor Hsp110 [73]. Similar findings were made with the aggregates of engineered β-sheet proteins that form amyloid-like inclusions [48].

Maintaining the solubility of a chronically expressed mutant protein, such as polyQ-expanded huntingtin, may divert considerable PN resources from the rest of the proteome. Indeed, expression of mutant huntingtin has been shown to interfere with the folding and conformational maintenance of endogenous (or exogenously expressed) metastable proteins [43-45], and with specific chaperone functions, such as the uncoating of clathrin cages in endocytosis[74]. Notably, due to its high degree of interconnectedness and inbuilt redundancy, the PN is robust and able to buffer the deleterious consequences of aberrant protein species for long periods of time (up to decades in humans). However, when the network becomes critically overloaded, it can no longer stabilize its metastable, aggregation-prone clients (Figure 3). Consequently, these proteins will increasingly populate non-native states that would normally be degraded but now accumulate as ubiquitylated species in aggregates [61]. When the network is overwhelmed, proteostasis collapse can be rapid and dramatic, as observed in the *C. elegans* model [33]. This loss of proteome stability contributes critically to cellular dysfunction and demise.

Mutations of PN components as cause for disease

The importance of proteostasis impairment in the pathogenesis of aggregate deposition disorders is reinforced by the existence of familial forms of neurodegeneration which are caused by mutation of PN components [75]. For

example, loss-of-function mutations in the ubiquitin ligase PARKIN and the PARKIN-related kinase PINK1 are the cause of early-onset PD [76, 77]. PARKIN cooperates with PINK1 in the ubiquitylation and selective autophagy of mitochondria (mitophagy). Defects in this pathway result in accumulation of damaged mitochondria, disturbances in calcium homeostasis and oxidative stress [78]. The motor neuron disease ALS can also be caused by mutations in PN components. Dominant mutations causing ALS have been mapped to several factors, including ubiquilin-2, a protein that recruits proteasome complexes to ubiquitylated proteins; sequestosome-1 (p62), a ubiquitin binding protein required for autophagy of aggregates; and VCP (p97/Cdc48), a AAA ATPase that functions in ERAD [79]. Marinesco-Sjoegren syndrome, a rare autosomal recessive disorder characterized by cerebellar ataxia, is caused by loss of function mutations of the HSPA5 cochaperone SIL1, which is required for protein translocation and folding in the endoplasmic reticulum [80, 81]. Mutations of the mitochondrial chaperonin Hsp60 cause autosomal dominant spastic paraplegia [82] and an autosomal-recessive neurodegenerative disorder linked to brain hypomyelination and leukodystrophy [83].

The PN as a target for pharmacological intervention

The physiological regulation of the PN is complex and involves several interconnected pathways, some of which are inducible by cellular stress. These signaling pathways regulate specific transcriptional programs to adjust proteostasis capacity at the level of protein synthesis, folding and degradation machineries. They include the cytosolic heat-shock response (HSR), the unfolded protein responses (UPR) of the ER and mitochondria, and additional pathways involved in inflammation and the responses to oxidative stress and caloric restriction [2]. The

complex mechanisms by which these forms of stress are sensed and the different signals integrated into a cellular response are only partially understood. For example, multiple signals from the cell membrane, cytosol, endomembrane system and nucleus feed into the cytosolic stress response that is executed by HSF-1 [54, 55]. In *C. elegans* the stress response pathways are also coordinated at the organismal level by cell non-autonomous mechanisms involving neuronal communication and humoral factors [84, 85]. There is also evidence that different cell types and distinct brain regions vary in their proteostasis capacity and their ability to respond to different forms of stress [36, 43, 86, 87]. A precise definition of these differences might help to explain why certain cell types are more vulnerable to aberrant protein folding than others, and why ubiquitously expressed proteins only aggregate in certain tissues.

Seeking ways to pharmacologically upregulate proteostasis capacity may open up new opportunities to combat neurodegeneration and other aggregate deposition diseases [1, 2]. This may be achieved by intervening at multiple sites of the PN or by using chemical chaperones that stabilize misfolded proteins. Induction of the cytosolic stress response by small molecule compounds or overexpressing members of the Hsp70 system has been shown to be beneficial in cells expressing different aggregation-prone proteins, to prevent the formation of toxic aggregates [69, 88-90] and increase the formation of (presumably less toxic) inclusion bodies [51]. The chemical chaperone 4-phenylbutyrate helps to restore ER proteostasis in metabolic diseases like type 2 diabetes [91], and upregulation of ER folding capacity by activating the ER stress response can improve the secretion of certain disease proteins such as mutant α1-antitrypsin and mitigate lysosomal enzyme deficiencies [92]. Induction of the ER stress factor XBP1s can even prevent amyloid-β neurotoxicity in model systems for AD [93]. Phramacologically prolonging the transient attenuation of

translation that occurs upon ER stress may be employed to adjust protein production rates to levels manageable by available chaperones [94]. Increasing proteolytic capacity provides an alternative approach to maintaining proteostasis. This may be achieved by induction of autophagy [95] or by inhibiting specific deubiquitinating enzymes, thereby increasing the rate at which the UPS clears misfolded proteins [96].

Beyond counteracting the toxic effects of aggregating disease proteins, enhancing proteostasis capacity also extends lifespan and preserves the responsiveness of model organisms to acute stress [97]. Conversely, the chronic presence of aggregates can suppress the ability of cells to adequately respond to stress [48], supporting the view that protein aggregation is a major driver of the aging process. Studies in mice showed that small molecule activators of the stress response, while effective early in disease, may lose efficacy during disease progression and aging [98]. On the other hand, sustained aggregate stress may result in a disproportionate response. For example, the long-term down-regulation of translation caused by chronic ER stress can be especially detrimental to neuronal cells [99, 100] which rely critically on ongoing translation for functionality. It will be crucial to unravel the mechanisms by which protein aggregation deregulates stress response pathways and undermines the cellular defense against toxic protein species. In any case, attempts to improve proteostasis pharmacologically would likely have to occur at an early stage of disease before the manifestation of severe cellular dysfunction.

Concluding remarks

Impairment of proteostasis is now being recognized as a basic mechanism by which chronic protein misfolding and toxic aggregation cause cellular dysfunction, facilitating the manifestation and progression of numerous neurodegenerative and

other aggregate deposition diseases. Due to the interconnected nature of the machineries of protein folding and degradation, deficiencies in specific PN components, for example as the result of their sequestration by aggregates, can have global detrimental effects. This sets in motion a self-propagating cycle that exacerbates proteome imbalance and eventually leads to proteostasis collapse and cell death. The gradual accumulation of proteome damage in post-mitotic tissues coupled with the age-dependent decline in proteostasis capacity and deregulation of stress response pathways can explain why age is the major risk factor for aggregate deposition diseases. Searching for effective ways to pharmacologically upregulate rate-limiting PN components may provide a viable strategy for therapeutic intervention. However, achieving this goal requires a detailed understanding of the organization and hierarchy of the PN and the signaling pathways underlying its control.

Figure 1: Self-propagating cycle of proteostasis decline in disease and aging.
Chronic expression of aberrantly folded protein species caused by disease, aging or external stress reduces proteostasis capacity by sequestering or otherwise inhibiting PN components. This results in misfolding and aggregation of endogenous proteins. These additional misfolded species in turn engage the proteostasis network (PN titration model), thereby reducing available proteostasis capacity further and driving a positive feedback loop that eventually leads to proteostasis collapse.

Figure 2: The proteostasis network (PN).
(A) The PN maintains protein homeostasis by controlling the levels of functional proteins and preventing the formation of toxic aggregates. This is achieved by integrating three branches of the PN: (1) protein synthesis, the chaperone pathways for the folding of newly-synthesized proteins and intracellular trafficking (PN branch of biogenesis; green), (2) the chaperone pathways for the remodeling of misfolded proteins and protein disaggregation (PN branch of conformational maintenance; blue), and (3) the pathways of protein degradation by the ubiquitin proteasome system (UPS) and autophagy (PN branch of degradation; red). Toxic aggregates (mainly diffusible, oligomeric states) may be converted to less toxic, insoluble inclusions of amorphous or fibrillar (amyloid-like) structure.
(B) Chaperones have important functions in all three branches of the PN. The approximate numbers of PN components in the human proteome are indicated. Adapted and modified from [3].

Figure 3: Mechanisms of proteostasis impairment.

(A) Homeostasis. The capacity of the chaperone network is sufficient to correctly fold the majority of newly synthesized proteins. The limited fraction of proteins that cannot be successfully folded upon synthesis (~5-10% of total) [101, 102] are degraded by the UPS, a process that may contribute to production of antigenic peptides [103].

(B) PN impairment by aggregate formation. Chaperone components are engaged and functionally depleted by soluble and insoluble protein aggregates (chaperone titration model). This prevents successful folding of endogenous chaperone clients and results in the accumulation of folding intermediates and misfolded states which are ubiquitylated and directed towards proteasomal degradation. When the accumulation of misfolded proteins exceeds the capacity of the UPS, additional protein aggregates containing ubiquitylated and non-ubiquitylated protein molecules form. These aggregates engage additional PN components, exacerbating PN impairment. Formation of insoluble inclusions may serve a protective role by binding less PN components.

(C) Acute vs. chronic stress. Eukaryotic cells contain multiple signaling mechanisms which respond to acute forms of conformational stress, such as the cytosolic heat stress response (HSR) and the unfolded protein response of the ER (UPR). Activation of these pathways by the accumulation of misfolded proteins results in rapid induction of PN components, especially chaperones and degradation machinery. At the same time, synthesis of the majority of other proteins is reduced, to free up PN capacity for the removal of misfolded proteins. However, this cellular state is only sustainable for a limited period of time, and if maintained for too long can result in apoptosis. Chronic stress, such as the constant production of protein aggregates during disease or aging, is thought to render cells stress-refractory, thereby enhancing proteome

imbalance.

Box 1: Huntington's disease

At least nine neurodegenerative diseases are associated with the expression of otherwise unrelated proteins containing polyglutamine (polyQ) expansion sequences. Among these, Huntington's disease (HD) is the most frequent affecting about 5-10 in 100,000 individuals. HD is associated with severe movement disorders and cognitive decline, and typically leads to death 15-20 years after the onset of symptoms. HD follows autosomal dominant inheritance and is caused by the expansion of a polyQ tract in the N-terminal exon of huntingtin, a ubiquitously expressed cytosolic protein of 3142 amino acids with unknown function. A characteristic feature of HD is the formation of detergent insoluble aggregates of mutant huntingtin in the nucleus and cytoplasm in a subset of neuronal cells in the cortex and striatum [104]. The length of the expanded polyQ stretch (38-100 Q and more) correlates with increased aggregation propensity [105] and is inversely correlated with the age of disease onset [106].

The formation of neuronal inclusions and toxic-gain-of-function pathology characteristic of HD can be reproduced in mouse models by expressing N-terminal fragments of huntingtin with an expanded polyQ tract [107]. Inclusions disappear when huntingtin expression is blocked [108]. Multiple lines of evidence suggest that polyQ expanded proteins titrate chaperones away from their clients, leading to proteostasis impairment [44, 74, 100, 109, 110]. Conversely, overexpression of various chaperones, such as members of the Hsp70 system, suppresses polyQ toxicity [3, 69, 75].

Box 2: The nucleus as quality control compartment

The proteostasis machinery in the nucleus differs from that of the cytosol in that no protein synthesis takes place in this compartment. In contrast to protein transport into the ER or mitochondria, the nuclear pore complexes allow the import of proteins in their folded and assembled states. Besides specific roles in histone remodeling, the nuclear chaperone machinery is therefore mainly involved in conformational protein maintenance and in the degradation of misfolded proteins. The nucleus is highly enriched in proteasome complexes [111] and contains specific ubiquitin ligases dedicated to quality control [112, 113]. During stress, import of most proteins into the nucleus is reduced but additional chaperones and proteasome complexes enter using specific import factors [114]. A substantial fraction of nuclear proteins are metastable. These proteins are conformationally destabilized upon stress and undergo proteasomal degradation [55].

Diseases associated with protein aggregation, such as HD, are often characterized by the presence of intranuclear inclusions. This may be explained by recent observations that misfolded cytosolic proteins, including mutants of huntigtin, are transported into the nucleus for proteasomal degradation [44, 71, 113, 115, 116]. When nuclear PN capacity is exhausted, misfolded proteins may form intranuclear inclusions. Since the autophagic machinery has no access to the nucleus, perhaps the only possibility to remove these aggregates is to transport them to the cytosol after disassembly of the nuclear envelope during mitosis. This would help to explain why postmitotic cells such as neurons are more vulnerable towards intranuclear inclusions.

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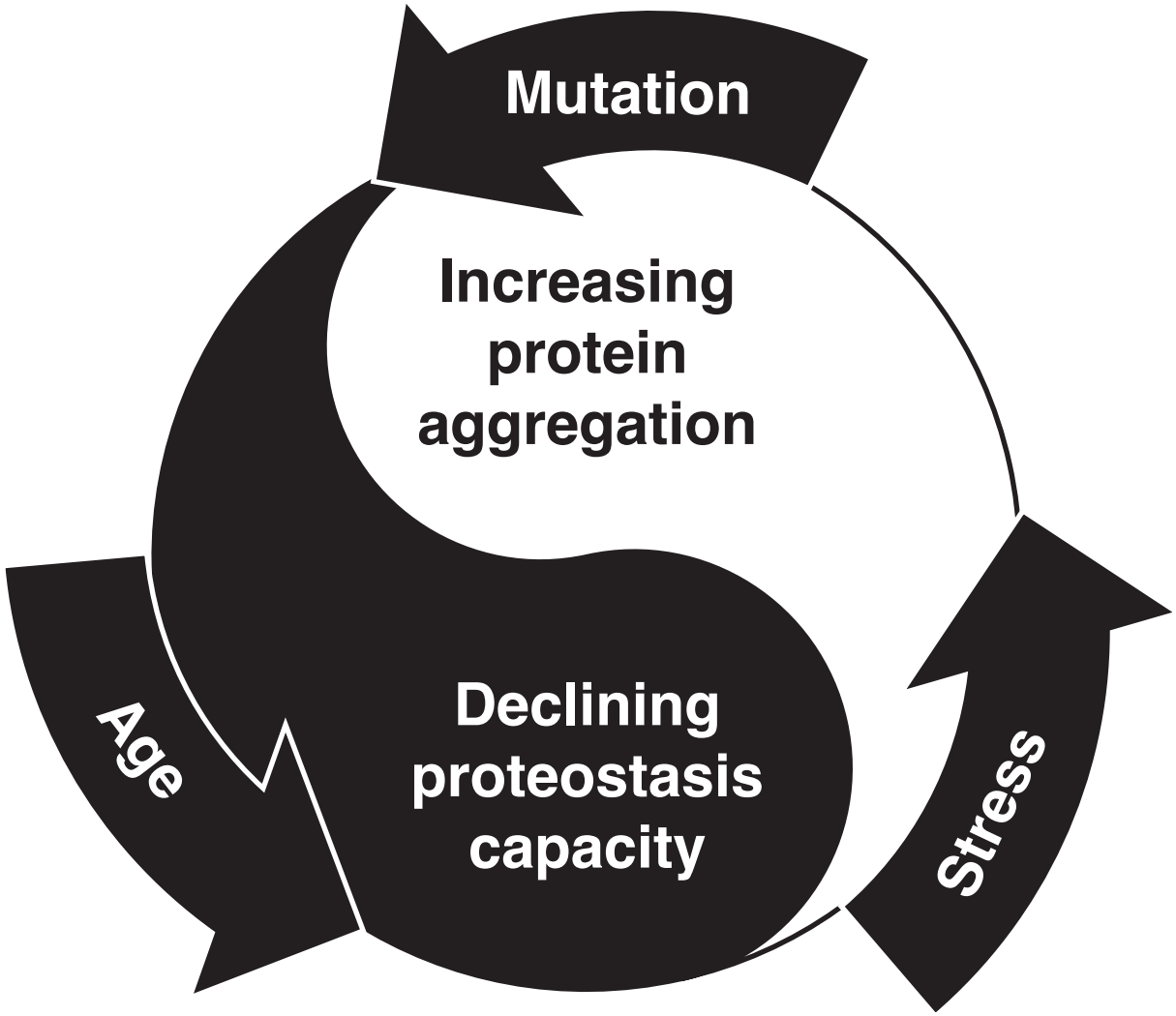
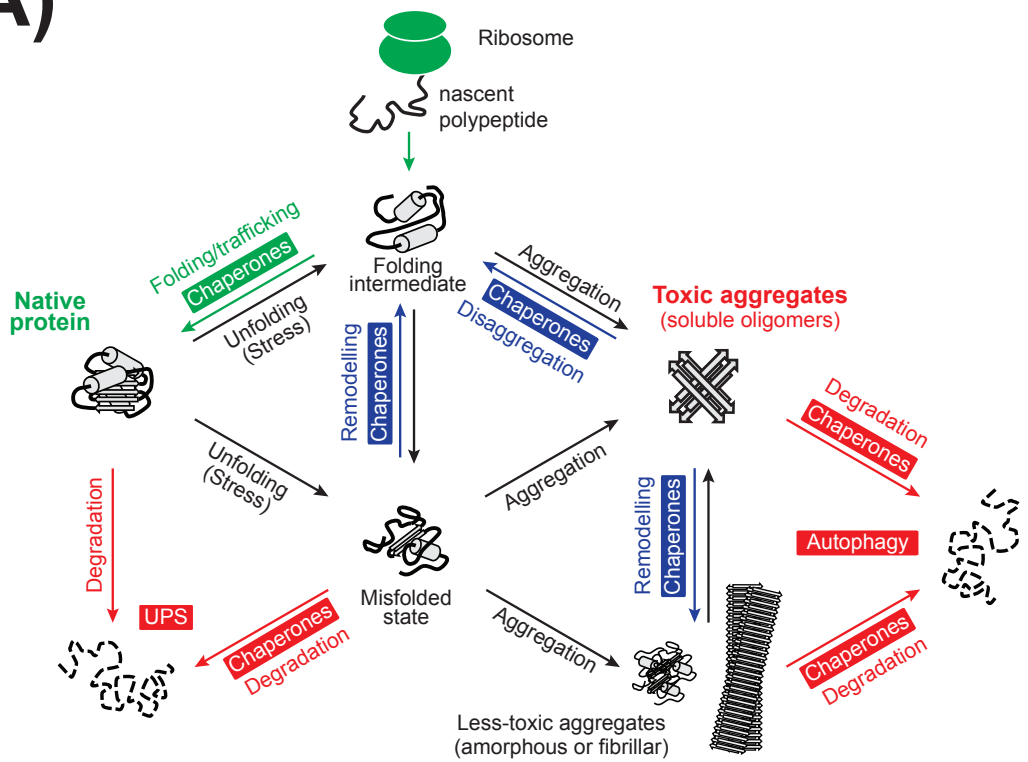

Mutation
Increasing protein aggregation
Stress
Declining proteostasis capacity
Age

(A)


Ribosome
nascent polypeptide
Folding intermediate
Native protein
Folding/trafficking
Chaperones
Unfolding (Stress)
Remodelling
Chaperones
Aggregation
Chaperones
Disaggregation
Toxic aggregates
(soluble oligomers)
Degradation
Chaperones
Unfolding (Stress)
Aggregation
Degradation
UPS
Misfolded state
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Degradation
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Degradation
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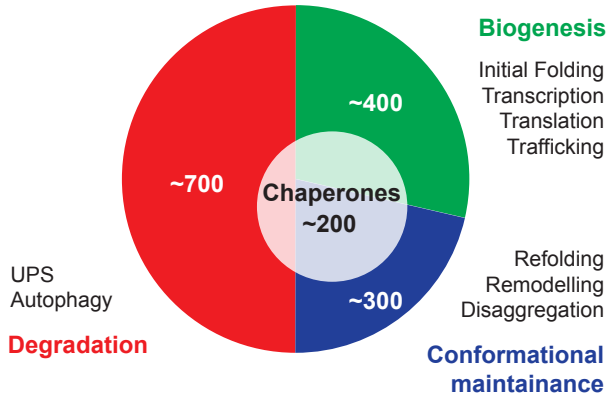
(amorphous or fibrillar)

(B)


Biogenesis
Initial Folding
Transcription
Translation
Trafficking
~400
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Chaperones
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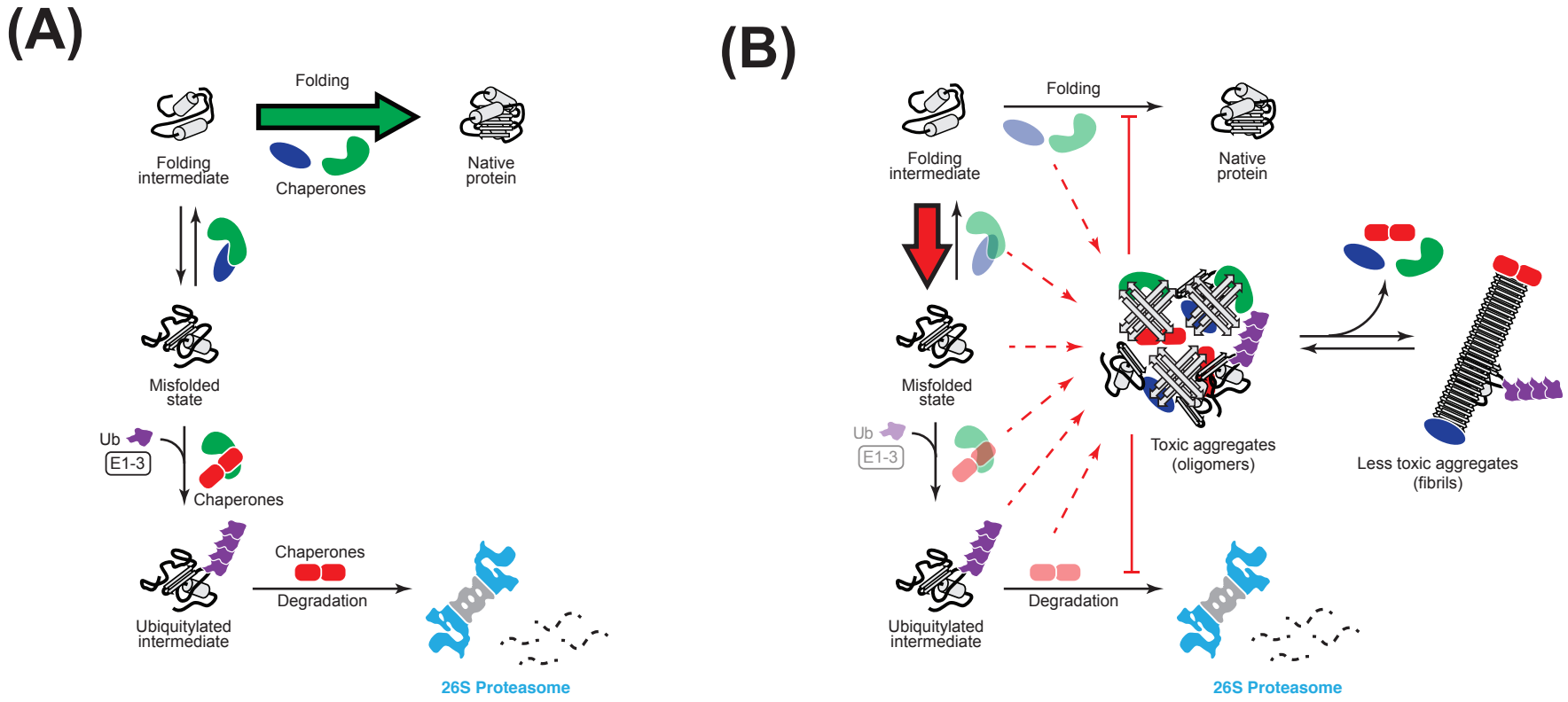
(A)
Folding
Folding intermediate
Native protein
Chaperones
Misfolded state
Ub
E1-3
Chaperones
Chaperones
Degradation
Ubiquitylated intermediate
26S Proteasome
(B)
Folding
Folding intermediate
Native protein
Misfolded state
Ub
E1-3
Toxic aggregates (oligomers)
Less toxic aggregates (fibrils)
Degradation
Ubiquitylated intermediate
26S Proteasome
(C)
STRESS
RESPONSE
Acute
STRESS
RESPONSE
Chronic