Pathways of cellular proteostasis in aging and disease

Courtney L. Klaips, Gopal Gunanathan Jayaraj, and F. Ulrich Hartl

Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany

Ensuring cellular protein homeostasis, or proteostasis, requires precise control of protein synthesis, folding, conformational maintenance, and degradation. A complex and adaptive proteostasis network coordinates these processes with molecular chaperones of different classes and their regulators functioning as major players. This network serves to ensure that cells have the proteins they need while minimizing misfolding or aggregation events that are hallmarks of age-associated proteinopathies, including neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. It is now clear that the capacity of cells to maintain proteostasis undergoes a decline during aging, rendering the organism susceptible to these pathologies. Here we discuss the major proteostasis pathways in light of recent research suggesting that their age-dependent failure can both contribute to and result from disease. We consider different strategies to modulate proteostasis capacity, which may help develop urgently needed therapies for neurodegeneration and other age-dependent pathologies.

Introduction

Human cells express more than ∼10,000 different proteins at any given time (Kulak et al., 2017), the majority of which must fold (and often assemble) to well-defined, three-dimensional structures to allow a myriad of cellular functions. Although the native conformation of a given protein is encoded by its amino acid sequence (Anfinsen, 1973), in the cell many proteins require assistance by molecular chaperones and other factors to fold efficiently and at a biologically relevant time scale (Balchin et al., 2016). Moreover, proteins often need to retain structural flexibility or contain significant unstructured regions to function, leaving them at risk of misfolding and aggregation (Chiti and Dobson, 2017). Even otherwise stably folded proteins may unfold and possibly aggregate under stress conditions, such as elevated temperatures. Finally, as proteins become terminally misfolded, or are no longer functionally required, they must be degraded to avoid damaging effects of their continued presence. Maintaining an intact proteome (proteostasis) thus requires not only strict control of the initial production and folding of a protein but also its conformational maintenance, control of abundance and subcellular localization, and finally, disposal by degradation.

A complex proteostasis network (PN) acts at each of these steps to maintain a balanced proteome linked by molecular chaperones of different classes as central players. These factors ensure de novo folding in a crowded cellular environment and maintain proteins in a soluble, nonaggregated state. Moreover, in conditions that disfavor folding or solubility, certain chaperones act to target misfolded proteins for degradation or spatial sequestration, thus protecting the rest of the proteome from aberrant interactions (Balchin et al., 2016; Sontag et al., 2017).

Here, we describe the major pathways of cellular proteostasis and outline the challenges they face during aging and disease. We exemplify these processes using mainly the proteostasis pathways operating in the cytosol, where most cellular proteins are produced. The major exceptions are the proteins associated with the endomembrane system and secretory proteins. These polypeptides generally fold and assemble in the ER. Although the environment of the ER is oxidizing and differs in several aspects from the reducing cytosol, the core principles governing overall proteostatic balance apply (Skach, 2009; Gidalevitz et al., 2013). Rather than focusing on specific disease states, we discuss common themes that have been shown to be relevant across multiple systems, suggesting a conserved and intimate linkage of proteostasis with the aging process and associated pathologies.

Organization of the PN

Because of the astronomically large number of possible conformations a polypeptide chain can adopt, the folding process is inherently error prone (Dobson et al., 1998; Bartlett and Radford, 2009). Production of misfolded proteins is further increased by stochastic errors of protein biogenesis occurring at the level of transcription and mRNA maturation and translation (Sachidanandam et al., 2001; Ng and Henikoff, 2006; Drummond and Wilke, 2008). Such failed protein products must be recognized and degraded to avoid aberrant interactions, making it a challenge to maintain a healthy proteome even under normal conditions. This challenge is exacerbated in the case of disease-associated mutations, environmental stress, and aging and if left unresolved can lead to the formation of toxic aggregate species. Thus, to maintain proteostasis, cells have evolved a wide variety of molecular chaperones and protein quality-control factors that are functionally linked with protein degradation machineries. This system is referred to as the PN (Balch et al., 2008; Fig. 1 A).

Correspondence to F. Ulrich Hartl: uhartl@biochem.mpg.de; Courtney L. Klaips: klaips@biochem.mpg.de

Abbreviations used: Hsp, heat-shock protein; PERK, protein kinase RNA-like ER kinase; PN, proteostasis network; sHsp, small Hsp; UPR, unfolded protein response; UPS, ubiquitin–proteasome system.

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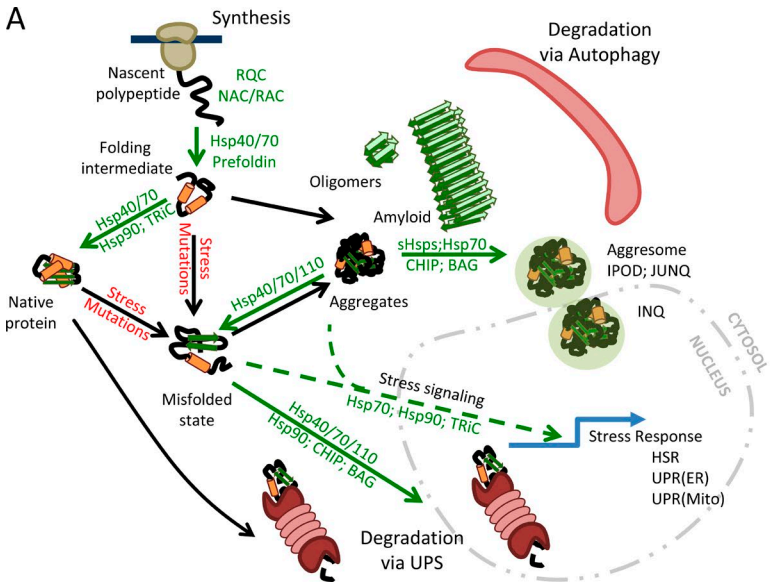


B

PN Branch	No. of components	Data Source
Synthesis	279	Rouillard et al., 2016
Folding (Chaperome)	332	Brehme et al., 2014
Degradation (UPS)	855	Garcia-Prat et al., 2016 Li et al., 2008; Nijman et al., 2005; Sowa et al., 2009
Degradation (Autophagy)	533	Garcia-Prat et al., 2016

Figure 1. **The PN.** (A) The flux of proteins through the PN relies on chaperones at many stages. PN capacity is sufficient to fold, often via intermediates, most newly synthesized polypeptides as they exit the ribosome. When a protein is no longer needed, it can be efficiently targeted for degradation either in the cytosol or nucleus via the UPS. Proteins that cannot be folded are also targeted for degradation via the UPS. During stress, the cell can increase PN capacity by activating a stress response. In aged or diseased cells, there is an increase in overall protein misfolding and aggregation, owing to increases in mutations and an overall decrease in PN capacity. These misfolded species may aggregate and/or be sequestered into large structures (aggresomes). A subset of misfolded species may form amyloid fibers that can further interfere with cellular processes. Chaperone factors that enhance particular steps are shown in green. Adapted from Hipp et al. (2014) and Kim et al. (2013). (B) Numbers for the major branches of the human PN, including synthesis, folding, and maintenance, and degradation branches, are shown. Datasets for generating these values were collected from the sources shown. The data were then arranged into nonoverlapping groups to represent the major PN branches. BAG, Bcl-2–associated athanogene; NAC, nascent chain-associated complex; RAC, ribosome-associated complex; RQC, ribosome quality control.

Defining the exact composition of the PN has proved difficult given the complexity of the human proteome. The PN has previously been proposed to consist of ∼1,000–1,400 components on the basis of our initial understanding of its organization (Balch et al., 2008; Powers et al., 2009; Kim et al., 2013; Hipp et al., 2014). Based on current annotations in databases and several large-scale genomic studies, we estimate that the PN comprises ∼2,000 factors that act in concert to maintain cellular proteostasis (Fig. 1 B). With increasing availability of functional annotations for the biological pathways in the human genome, these numbers will be further refined.

Operationally, the PN can be divided into three branches composed of factors belonging to major processes: (1) protein synthesis, (2) folding and conformational maintenance (often coupled to transport and/or assembly), and (3) protein degradation (the ubiquitin–proteasome system [UPS] and autophagy–lysosome system; Fig. 1). Molecular chaperones and their regulatory cofactors act as liaisons connecting all these processes.

A set of ∼280 components participate directly in nascent polypeptide chain synthesis (Wolff et al., 2014; Rouillard et al., 2016; Fig. 1 B). Apart from the core constituents of the translational machinery, several chaperones act on the ribosome to prevent premature misfolding of the nascent chain and assist in cotranslational folding. Quality-control factors of the UPS interface with protein synthesis to remove defective and stalled nascent chains as part of ribosomal quality-control pathways (Brandman and Hegde, 2016).

Newly synthesized proteins may fold cotranslationally or may rapidly complete their folding on release from the ribosome. Folding, and in some cases assembly to oligomeric complexes, is mediated by molecular chaperones, often involving sequential interactions with members of different chaperone classes (Langer et al., 1992; Frydman et al., 1994; Balchin et al., 2016). The repertoire of human chaperones (the "chaperome") contains ∼330 members of several functionally distinct gene families, which cater to diverse substrate clients (Brehme et al., 2014; Sala et al., 2017; Fig. 1 B).

Misfolded and aggregated proteins must be removed from the system by proteolytic degradation to prevent the accumulation of toxic species. Eukaryotic cells invest extensively in protein degradation machineries, with the two major pathways of the UPS and autophagy comprising ∼850 and ∼500 different components, respectively (Nijman et al., 2005; Li et al., 2008; Sowa et al., 2009; Varshavsky, 2012; García-Prat et al., 2016; Fig. 1 B). The UPS mainly serves to target individual proteins to the proteasome, whereas the autophagy system clears larger aggregates or membrane-associated proteins (Menzies et al., 2015; Ciechanover and Kwon, 2017).

These branches of the PN are functionally coordinated by various signaling cascades, which sense and respond to imbalances in proteostasis (Fig. 1 A). In this way, cells constantly monitor and adjust their proteome status in response to internal and external changes. The PN not only enables this adjustment, but is itself adaptive to the needs of specific cell types. In "simpler" organisms such as yeast, the basic organization of the PN may be rather constant, only tuning itself to fluctuations in environmental conditions. However, in metazoans (Guisbert et al., 2013), especially in complex mammalian systems (Uhlén et al., 2015), tissue-specific proteomes and regulatory programs imply that there must be a marked heterogeneity in aspects of proteostasis across diverse cell types, suggesting the existence of tissue-specific PNs (Sala et al., 2017) with differing contributions of the three branches.

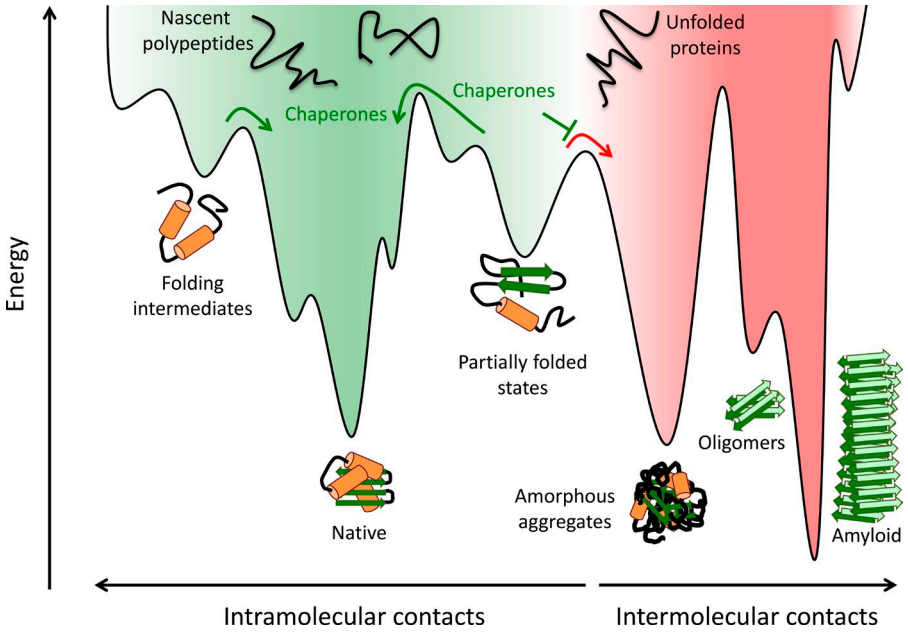


Figure 2. **Protein folding and aggregation.** Nascent polypeptides fold by sampling various conformations and sequestering hydrophobic amino acid residues. Partially folded intermediates, both on- and off-pathway, can become trapped in localized energy minima. These species are at risk of aggregation by forming aberrant intermolecular contacts, which can lead to the formation of oligomers, amorphous aggregates, and amyloid fibrils. Molecular chaperones promote the formation of the native species by lowering free-energy barriers between kinetically stable intermediates, smoothing the protein folding landscape (green arrows), and preventing aberrant intermolecular interactions (red arrow). Adapted from Balchin et al. (2016), Hartl et al. (2011), and Kim et al. (2013).

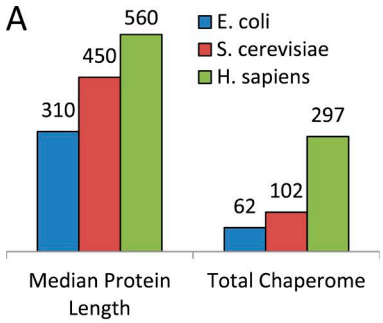
Many diseases, including type II diabetes and the major neurodegenerative pathologies, are associated with a reduced function of the PN, which may be caused by mutations in PN components (Kakkar et al., 2014) or by interference of toxic aggregate species with PN function (Hipp et al., 2014). Importantly, as shown in model organisms, aging is also associated with a general decrease in PN capacity and a corresponding increase in protein aggregation, which manifests as a functional decline in many cellular pathways (Taylor and Dillin, 2011; Labbadia and Morimoto, 2015a). In the following sections, we discuss the roles of the major pathways within the PN.

Protein synthesis. Although the production of individual proteins is regulated by specific factors and pathways, the levels of bulk protein synthesis must be adjusted to the protein folding capacity of the cell to avoid the accumulation of misfolded proteins. Indeed, in key lifespan extension pathways such as caloric restriction, increased proteostasis capacity is conferred, at least in part, by a general decrease in protein translation (Hansen et al., 2007; Taylor and Dillin, 2011). Translation attenuation is also critical in relieving PN overload in conditions of conformational stress. This is typically mediated by inhibition of translation initiation factor 2α (eIF2α). For example, on activation of the unfolded protein response (UPR) to the accumulation of misfolded proteins in the ER, protein kinase RNA-like ER kinase (PERK) in the ER membrane phosphorylates eIF2α, thereby attenuating its function in translation (Harding et al., 2001).

Protein folding and aggregation. Polypeptide chains fold by sequestering hydrophobic residues and forming stabilizing intramolecular interactions to achieve a low free-energy state (Fig. 2). Rather than sampling all potential folding states, a process that would take an insurmountable amount of time, polypeptides proceed toward their native conformation by increasingly forming local and long-range contacts between amino acid residues, thereby limiting the conformational space that must be explored (Dinner et al., 2000; Bartlett and Radford, 2009). In this way, many small proteins can achieve their proper fold quickly and efficiently in vitro. However, once placed in the highly crowded cellular environment, proteins often face significant challenges during folding, because partially folded states with exposed hydrophobic amino acids residues are in danger of misfolding and aggregating. Aberrant folding may occur during de novo synthesis or in conditions of conformational stress, where preexisting proteins may fail to maintain their folded states. Destabilizing mutations or the presence of intrinsically unstructured regions can also predispose polypeptides to misfolding (Dunker et al., 2008; Gershenson et al., 2014).

Unlike in vitro folding studies (Anfinsen, 1973; Bartlett and Radford, 2009), which start from complete proteins, in the cell proteins are synthesized vectorially on the ribosome, which means that the structural information necessary for folding becomes available gradually and not all at once. Translation is slow relative to rates of folding, allowing for the possibility of partial structure formation, both native or misfolded, before the completion of protein synthesis. Cotranslational folding limits the amount of time nascent chains populate potentially vulnerable, nonnative states (Balchin et al., 2016). Some very small proteins (~50 amino acids in length) even fold to completion within the widening exit portal of the ribosome (Holtkamp et al., 2015; Marino et al., 2016). However, the major part of the ribosomal exit channel is too narrow to allow structure formation (Wilson and Beckmann, 2011), and thus the nascent chains of larger proteins must first emerge from the ribosome before they can fold, which puts them at risk of misfolding and aberrant interactions. The ribosomal surface may influence the folding process (Kaiser et al., 2011; Cabrita et al., 2016). Moreover, the topology of ribosomes in the context of polysomes, where translating ribosomes may approach each other closely, is optimized to reduce the risk of interactions between nascent chains. The ribosomes adopt a staggered "pseudohelical" arrangement, in which their polypeptide exit sites are at maximal distance from each other (Brandt et al., 2009). Multidomain proteins often fold their domains sequentially during translation, thereby avoiding nonnative interactions between concomitantly folding domains (Netzer and Hartl, 1997; Frydman et al., 1999).

Average proteome and protein sizes have increased dramatically during evolution from bacteria to eukarya (Balchin et



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	E. coli	S. cerevisiae	H. sapiens
sHSP	2	7	10
HSP60	1	9	14
HSP70	3	14	17
HSP40	6	22	49
HSP90	1	2	5
Cofactors	0	11	41

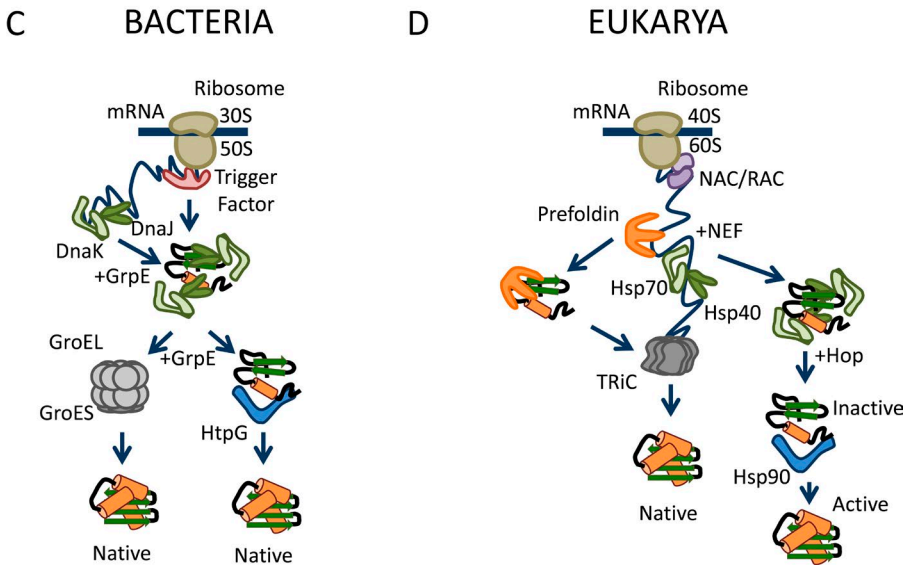

Figure 3. **Proteome complexity increases from prokaryotes to eukaryotes.** (A) The average protein length (in number of amino acids) and total number of chaperones in the proteomes of bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*), and humans (*Homo sapiens*) are shown. The increase in proteome complexity with increasing organismal complexity, from prokaryotes to eukaryotes, and from single-cell to multicellular organisms is accompanied by an increase in chaperone number. Comparative chaperomes were generated by UniProtKB queries by using identical keyword searches for chaperones for each organism. Differences between the total human chaperome number and previously reported numbers (Brehme et al., 2014; Fig. 1 B) arise from a more-stringent definition here to ensure appropriate comparisons between organisms. (B) The numbers of members of each of the major chaperone classes are shown for different organisms with selective cochaperones shown for comparison (Genevaux et al., 2007; Vos et al., 2008; Brehme et al., 2014; Rizzolo et al., 2017). Protein folding in the bacterial (C) and eukaryotic (D) cytosol. Most proteins use folding assistance on exit from the ribosome. Ribosome-associated factors include Trigger factor in bacteria and the nascent chain-associated complex (NAC) and ribosome-associated complex (RAC) in eukaryotes. Downstream Hsp70s (DnaK in bacteria) work with their cofactors Hsp40 (DnaJ in bacteria) and nucleotide exchange factors (NEFs; GrpE in bacteria) in protein folding. Some proteins must be transferred to the chaperonin class of chaperones for further folding (GroEL/ES in bacteria and TRiC in eukaryotes). In eukaryotes, prefoldin can transfer some substrates directly to TRiC. Cofactors such as Hop (Hsp70-Hsp90 organizing protein) can mediate interactions with Hsp90 (HtpG in bacteria), which also acts downstream of Hsp70 in the folding of a subset of proteins mainly engaged in cell signaling. Adapted from Balchin et al. (2016).

al., 2016; Fig. 3 A). Proteins of more than ∼100 amino acids in length constitute the vast majority of proteins in all domains of life and typically fold via intermediate states with incompletely buried hydrophobic residues (Dinner et al., 2000; Brockwell and Radford, 2007; Fig. 2). Such intermediates may be kinetically stable and may be highly aggregation prone (Gershenson et al., 2014), particularly in the crowded environment of the cell (∼300 g of protein per liter), where macromolecular interactions are enhanced compared with dilute solution (Ellis and Minton, 2006). Although the majority of resulting aggregates are amorphous (i.e., lacking long-range structural order), a subset of mostly smaller proteins, often containing unstructured regions (Dunker et al., 2008), can form ordered fibrillar aggregates, referred to as amyloid or amyloid-like, and which are characterized by β-strands running perpendicular to the fibril axis (cross-β-structure; Chiti and Dobson, 2017; Fig. 2). Such amyloid aggregates form insoluble deposits and are the hallmark of several age-dependent proteinopathies, including Alzheimer's, Parkinson's, and Huntington's diseases (Ross and Poirier, 2004). More proteins transition to an insoluble, aggregated state when they exceed their normal cellular abundance or when imbalances occur between subunits of oligomeric complexes (Vendruscolo et al., 2011; Ciryam et al., 2013; Chiti and Dobson, 2017), a phenomenon that becomes more prevalent during aging, as shown in the nematode *Caenorhabditis elegans* (Walther et al., 2015).

Molecular chaperones—central organizers of the PN. To overcome the challenges to protein folding and solubility, cells have evolved molecular chaperones (Fig. 3 A). We define a molecular chaperone as a protein that assists in the folding, assembly, conformational maintenance, or regulation of another protein without becoming part of its final structure (Hartl, 1996). The chaperones that participate broadly in de novo folding recognize generic structural features of nonnative proteins, primarily exposed hydrophobic amino acid residues, and promote folding by kinetic partitioning of nonnative states (Kim et al., 2013; Fig. 2). Many chaperones are induced under conditions of stress, such as heat shock, and in addition to their functions in de novo folding are also involved in protein refolding, disaggregation, oligomeric assembly, trafficking, and degradation (Balchin et al., 2016; Fig. 1 A). Although the core chaperone machineries (heat-shock protein [Hsp] 70s, Hsp90s, chaperonins, and small Hsps [sHsps]) are already present in prokaryotes, a strong expansion in the number of their regulatory cofactors (Hsp40s, tetratricopeptide repeat proteins) is notable as eukaryotic proteomes increase in complexity (Genevaux et al., 2007; Vos et al., 2008; Brehme et al., 2014; Rizzolo et al., 2017; Fig. 3 B).

A first tier of chaperones interacts directly with the ribosome close to the polypeptide exit site. These components are typically not stress-inducible (Albanèse et al., 2006) and include Trigger factor in bacteria and specialized chaperone complexes, such as nascent chain-associated complex and ribosome-associated complex, in eukaryotes (Fig. 3, C and D). They interact with exposed hydrophobic sequences of the emerging nascent chain and act to prevent premature (mis)folding, maintaining the polypeptide in a nonaggregated, folding competent state until sufficient structural elements for productive folding are available (Agashe et al., 2004; Kaiser et al., 2006; Preissler and Deuerling, 2012; Nilsson et al., 2016). Although most proteins may only require these chaperones to achieve their native fold, proteins with complex domain topologies and multidomain proteins need assistance by additional chaperone classes that act downstream.

Such proteins may next interact cotranslationally or posttranslationally with a member of the Hsp70 chaperone family (DnaK in prokaryotes; Fig. 3, C and D), a ubiquitous class of ATP-dependent chaperones of ∼70 kD with a hub position in the PN. The Hsp70 C-terminal domain binds short hydrophobic peptide sequences of about seven residues that are exposed by nascent and nonnative protein substrates (Rüdiger et al., 1997; Mayer et al., 2000; Clerico et al., 2015). The affinity of the C-terminal domain for protein substrate is allosterically regulated by ATP binding and hydrolysis in the N-terminal ATPase domain. Hsp70s rely on regulatory chaperone cofactors of the Hsp40 class (also known as J-proteins), which typically bind first to exposed hydrophobic patches on nonnative proteins and recruit Hsp70 (Kampinga and Craig, 2010; Nillegoda et al., 2017). These factors then stimulate the hydrolysis of Hsp70-bound ATP, thereby catalyzing the closing of the Hsp70 peptide binding cleft (Clerico et al., 2015). There are ∼50 different Hsp40 proteins in human cells (Fig. 3 B), which fall into three structural subtypes and have different subcellular localizations (Kampinga and Craig, 2010). They confer broad functionality to the Hsp70 system, allowing these chaperones not only to participate in the initial folding of nascent chains but also in conformational maintenance (Mashaghi et al., 2016), disaggregation (Diamant et al., 2000; Ben-Zvi et al., 2004; Rampelt et al., 2012), and the targeting of terminally misfolded proteins for degradation (Kettern et al., 2010). Nucleotide exchange factors are necessary to allow Hsp70 to perform cycles of substrate binding and release (Laufen et al., 1999; Mayer and Bukau, 2005; Winkler et al., 2012).

Proteins that are unable to fold through such Hsp70 cycles may be transferred to the chaperonin class of chaperones (Hsp60s), which includes GroEL/GroES in bacteria, Hsp60 in mitochondria, and TRiC/CCT in the eukaryotic cytosol (Fig. 3, C and D). These chaperonin proteins form multimeric, cylindrical complexes that function by transiently encapsulating individual nonnative proteins so they can fold, unimpaired by aggregation (Lopez et al., 2015; Hayer-Hartl et al., 2016). The opening and closing of the folding chamber is regulated by the ATPase of the chaperonin, either in conjunction with a separate lid-like cofactor of the GroES-type (for GroEL and mitochondrial Hsp60) or lid-structure built into the chaperonin complex (for TRiC/CCT). Although only ∼10% of the proteome requires a chaperonin to fold, substrates include essential and highly abundant proteins, such as actin and tubulin. Accordingly, deletion or mutation of TRiC is toxic and has been implicated in disease. Neurodegenerative disorders affecting myelination, spastic paraplegia, and leukodystrophy are caused by autosomally inherited mutations in mitochondrial Hsp60 (Hansen et al., 2002; Magen et al., 2008).

The highly conserved Hsp90 chaperone system also functions downstream of Hsp70 in maintaining a variety of signaling pathways via the folding and conformational regulation of their signal-transduction molecules (Sharma et al., 2012; Taipale et al., 2012). Hsp90 is active as a homodimer and mediates protein folding via ATP-dependent structural changes in cooperation with a multitude of cofactors (Wandinger et al., 2008). Hsp90 can bind substrates that are near native, thereby stabilizing metastable clients, such as kinases and steroid receptor molecules, in a conformation poised for activation by ligand binding (Fig. 1 A and Fig. 3, B and C). Because of its role in the folding of many disease-relevant proteins, pharmacologic inhibition of Hsp90 is being considered as a strategy in the treatment of many diseases from cancer to viruses (Whitesell et al., 1994; Balch et al., 2008; Geller et al., 2013; Mbofung et al., 2017).

Maintaining the metastable proteome. After initial folding, many proteins continue to require chaperone surveillance to maintain their functional form. Proteins are often active under conditions just at the cusp of stability, and their functional conformational states may be challenged under stress conditions. Additionally, many proteins contain intrinsically unstructured regions or sequences of low amino acid complexity important for their function, including up to 75% of signaling molecules in mammalian cells (Dunker et al., 2008). These proteins may acquire a stable structure only on binding to a ligand or other macromolecular surface. It has become clear in recent years that a hallmark of cellular aging is a gradual loss of proteome balance and accumulation of protein aggregates. This is thought to be due to at least in part an increase in the accumulation of errors in translation, splicing, or molecular misreading and to an increased production of oxidized and carbonylated proteins (Aguilaniu et al., 2003; López-Otín et al., 2013). The proteins of the PN are not exempt from such modifications. Indeed, studies in *C. elegans* and other model systems have shown that aged organisms have a markedly reduced ability to maintain metastable proteins in their soluble states (Morley et al., 2002; David et al., 2010; Gupta et al., 2011; Walther et al., 2015). In worms, this decline in PN capacity is tied to development, suggestive of a regulated program of aging (Ben-Zvi et al., 2009; Labbadia and Morimoto, 2015b).

A healthy chaperone network is thus required to maintain the metastable proteome and to prevent the accumulation of toxic aggregate species. The Hsp70 system and the so-called sHsps, the latter functioning as multidisperse chaperone oligomers (Haslbeck et al., 2005), are particularly important in this regard. Upon acute stress, such as heat exposure, 10–30% of cytosolic proteins are potential clients of sHsps, indicating an important role in the maintenance of proteome stability (Haslbeck et al., 2004; Mymrikov et al., 2017). When the system is overburdened, however, misfolded species will form and may aggregate. In these cases the association of sHsps with the aggregates themselves has been shown to aid in their resolution by the cell during recovery from conformational stress. Association of sHsps and chaperone cofactors with aggregates enables downstream processing (Ben-Zvi et al., 2004; Malinovska et al., 2012; Rampelt et al., 2012; Żwirowski et al., 2017) and eventual disaggregation by Hsp70/Hsp40/Hsp110 machineries (Mogk et al., 2003; Nillegoda and Bukau, 2015). In yeast, the disaggregation capacity of cells is further enhanced by a

specialized AAA+ ATPase called Hsp104, which along with its Hsp70/Hsp40 cofactors has been shown to disaggregate many amyloid aggregates (Parsell et al., 1994; Glover and Lindquist, 1998; Wegrzyn et al., 2001).

Disposal by degradation. Proteins that are unable to fold or refold, despite intervention by chaperones, must be disposed of to prevent the accumulation of potentially toxic aggregate species. Such terminally misfolded proteins undergo proteolytic degradation mainly by the UPS (Varshavsky, 2012; Ciechanover and Kwon, 2017) or by chaperone-mediated lysosomal degradation (Kettern et al., 2010; Cuervo and Wong, 2014). The Hsp70 and Hsp90 chaperone systems are intimately involved in these processes, because the E3 ubiquitin ligase Chip binds the C terminus of these chaperones and ubiquitylates misfolded chaperone-bound proteins (Esser et al., 2004; Fig. 1 A). As shown recently, surplus proteins that fail to assemble with their partner molecules are recognized by a specific E3 ligase (UBE20; Yanagitani et al., 2017).

A subset of proteins that misfolds in the cytosol undergoes chaperone-mediated transport into the nucleus to be degraded by nuclear proteasomes (Heck et al., 2010; Prasad et al., 2010; Park et al., 2013; Shibata and Morimoto, 2014; Fig. 1 A). Most of the proteins known to use this pathway are ectopically expressed secretory proteins. The extent to which endogenous, misfolded proteins are degraded in the nucleus remains to be established. Given that an abundance of proteasomes is found in the nucleus (Russell et al., 1999; von Mikecz, 2006), it is tempting to speculate that compartmentalizing synthesis/folding and degradation provides an evolutionary advantage by preventing premature degradation. The same principle would apply to the process of ER-mediated degradation, wherein misfolded proteins are retrotranslocated from the ER to the cytosol for disposal by the proteasome (Vembar and Brodsky, 2008).

Aggregates may be resolved by the Hsp70/Hsp40/Hsp110 machinery before transfer into the proteasome (Hjerpe et al., 2016). Certain aggregate species resistant to disaggregation may be cleared directly by selective autophagy and lysosomal degradation (Lamark and Johansen, 2012), processes that also target a variety of additional substrates including membrane bound organelles (Mizushima, 2007).

Many cell types show a decline in UPS activity and autophagy during aging (Rubinsztein et al., 2011; Cuervo and Wong, 2014), contributing to the widespread protein aggregation that is observed in postmitotic cells, such as muscle and neurons, and predisposing the latter for certain neurodegenerative diseases (David et al., 2010; Hamer et al., 2010; Walther et al., 2015). Because disease-associated proteins tend to be metastable, a slight increase in their abundance as clearance systems decline can have dramatic effects on their aggregation propensity (Ciryam et al., 2013; Kundra et al., 2017). Aging cells are also less able to cope with and dispose of amyloid-like aggregates (Morley et al., 2002), as exemplified by the fact that cellular aggregate deposits persist although they are typically associated with ubiquitin (Lowe et al., 1988; Bence et al., 2001; Waelter et al., 2001). These aggregates often sequester important components of the PN, which leads to further proteostatic impairment with buildup of damaged protein species and increased risk of aggregation (Bennett et al., 2005; Hipp et al., 2014; Itakura et al., 2016).

Compartmentalization of damaged proteins. If attempts to prevent, refold, or degrade aberrant protein species fail, a final line of defense against their interference with cellular processes is their controlled sequestration into more benign aggregate deposits or inclusion bodies (Sontag et al., 2017). Depending on the properties of the misfolded proteins and their ability for eventual resolubilization, such deposits can be directed to several different localizations within the cytosol or nucleus and are referred to as an IPOD (for insoluble protein deposit), JUNQ (for juxtanuclear quality-control compartment), or INQ (for intranuclear quality-control compartment) in yeast and as an aggresome in mammalian cells (Johnston et al., 1998; Kaganovich et al., 2008; Miller et al., 2015; Fig. 1 A). Their formation is itself dependent on several quality-control components including chaperones (Malinovska et al., 2012; Escusa-Toret et al., 2013; Wolfe et al., 2013). In addition to providing an environment in which aggregates may be shielded and thus prevented from engaging in potentially toxic interactions, in dividing cells the inclusions also serve as a way to minimize the amount of aberrant proteins that are passed on to daughter cells (Hill et al., 2017). Like other proteostasis pathways, the ability of a cell to maintain spatial quality control also declines with age (Escusa-Toret et al., 2013), and cells that lack this ability show accelerated aging (Erjavec et al., 2007).

Toxicity caused by aggregation

Proteins have an intrinsic capacity to convert from their native state to intractable fibrillar aggregates, but under normal physiological conditions this tendency is resisted by cellular proteostasis mechanisms (Chiti and Dobson, 2017). However, the propensity to form amyloid-like aggregates is more pronounced for certain metastable proteins, including those associated with disease, especially when exceeding the cellular concentrations at which they are soluble (Ciryam et al., 2013). Dysregulation of protein abundance and protein stoichiometries may occur in an age-dependent manner, as observed in nematodes and other model organisms (Walther et al., 2015). Indeed, recent research shows that the formation of insoluble protein deposits in neurodegenerative syndromes such as Alzheimer's disease occurs concomitantly with the aggregation of a large set of highly expressed and aggregation-prone proteins that constitute a metastable subproteome (Kundra et al., 2017). The metastable subproteome includes many RNA-binding proteins that contain unstructured or low-complexity sequences. As shown recently, such proteins often have the ability to undergo liquid–liquid phase transitions (Feric et al., 2016), forming dynamic droplet-like compartments in the nucleus and cytosol that participate in RNA metabolism, ribosome biogenesis, cell signaling, and other processes (Banani et al., 2017). However, the normally dynamic behavior of these condensates is highly sensitive to changes in the physicochemical environment of cells, and aberrant phase transition behavior, leading to fibril formation, has been linked with aging and diseases such as amyotrophic lateral sclerosis (Alberti and Hyman, 2016). These recent observations help explain how the age-dependent decline in protein homeostasis favors the stochastic manifestation of neurodegenerative aggregation. Importantly, even in dominantly inherited neurodegenerative disorders, such as those caused by expanded polyglutamine sequences (Scherzinger et al., 1999; Gusella and MacDonald, 2006), manifestation is age dependent and triggered by PN decline (Morley et al., 2002; Gidalevitz et al., 2006).

Aggregation in disease typically causes gain-of-function toxicity, which means that the cytotoxic effects are largely unrelated to the normal function of the disease protein (Ross and Poirier, 2004; Chiti and Dobson, 2017). However, the presence

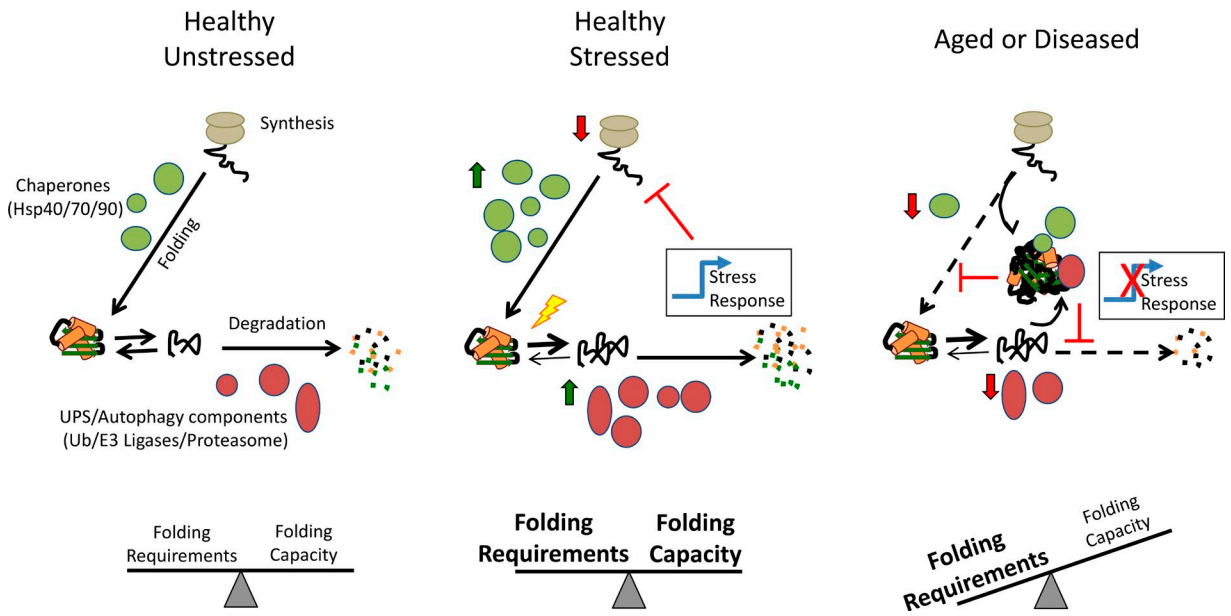

Figure 4. **Healthy and aged proteostasis.** (Left) The PN ensures that most proteins fold to a stable native state. When these proteins are no longer needed or errors in folding occur, they are efficiently targeted for degradation, primarily via the UPS. On transient stress (middle), otherwise healthy cells activate a stress response. Chaperone sensors bind to misfolded species and trigger the appropriate transcriptional program leading to a general increase in protein-folding capacity, increase in protein turnover, and decrease in the production of additional substrates via attenuation of general protein translation. In cases of chronic stress, such as during disease or aging (right), sequestration of key PN components by aggregates can lead to aberrant transcriptional programs, a deficit in folding capacity due to a lack of functionally available chaperones, and a buildup of misfolded species due to a decline in proteasomal capacity. This can lead to a chronic inability to restore PN balance and further accumulation of misfolded species.

of large fibrillar aggregate deposits does not always correlate with disease onset or severity (Kayed et al., 2003; Leitman et al., 2013; Chiti and Dobson, 2017). Indeed, work over the past years revealed that the most toxic aggregate species may be soluble oligomers and small insoluble species with little or no fibrillar content (Chiti and Dobson, 2017). Such oligomers expose hydrophobic residues and unpaired polypeptide backbone structures, features that render them highly interactive with other proteins, including proteins enriched in low-complexity sequences and critical factors of the PN, and with membranes (Kayed et al., 2003; Olzscha et al., 2011; Winner et al., 2011; Park et al., 2013; Kim et al., 2016b; Woerner et al., 2016).

Although the exact nature of the most-toxic species remains a subject of discussion, in many cases the larger amyloid aggregates may exert a relative protective effect by sequestering the more-toxic oligomers and by having a reduced surface-to-volume ratio (Saudou et al., 1998; Arrasate et al., 2004; Douglas et al., 2008; Kim et al., 2016b). However, key cellular factors, including PN components, that interact aberrantly with soluble oligomers may also be sequestered in the insoluble deposits, contributing to cellular dysfunction (Olzscha et al., 2011; Park et al., 2013; Hipp et al., 2014; Ripaud et al., 2014; Yu et al., 2014; Woerner et al., 2016). Moreover, large intracellular aggregate deposits sterically displace membrane structures and may cause their fragmentation, as recently shown for inclusions of polyglutamine expansion proteins by cryo-electron tomography (Bäuerlein et al., 2017). In many model systems, exogenous expression of individual chaperone components, such as Hsp70, or up-regulation of multiple chaperones by pharmacologic induction of the stress response has been shown to either prevent toxic aggregation or to direct the formation of less-toxic but still aggregated species (Muchowski et al., 2000; Sittler et al., 2001; Holmes et al., 2014; Nagy et al., 2016).

Stress response pathways

Although the protein quality-control networks ensure proteostasis under basal conditions, on conformational stress, such as increases in temperature or exposure to oxidative agents, many additional proteins become prone to misfolding, with proteins comprising the metastable subproteome being particularly vulnerable. Cells adapt to such conditions by activating stress-response pathways to increase PN components, decrease substrate load, and resolve misfolded or aggregated species (Fig. 4). In metazoans the stress-response pathways additionally underlie cell nonautonomous regulation, allowing coordination within and between tissues and organs (Taylor et al., 2014; Sala et al., 2017).

The cytosolic stress response is regulated primarily by heat-shock transcription factor 1 (Hsf1), which is maintained in an inactive state by association with chaperones including Hsp90 (Zou et al., 1998) and Hsp70 (Zheng et al., 2016). The current model suggests that on heat stress, these chaperones are titrated away from Hsf1 by binding to denatured proteins. Hsf1 is then free to induce the transcription of a wide range of proteostasis components (Zou et al., 1998; Zheng et al., 2016), although general protein translation is decreased, reducing the load on the chaperone machinery. Concurrently, expression of chaperones and other quality-control elements, such as proteasomal components, is increased to prevent and resolve misfolded proteins and aggregation. Finally, once the stressor is removed, a negative-feedback loop on Hsf1 activity ensures a return to stasis within the system (Akerfelt et al., 2010; Gomez-Pastor et al., 2017).

Similar stress-response pathways include the UPR in the ER and mitochondria (Walter and Ron, 2011; Jovaisaite et al., 2014; Schulz and Haynes, 2015; Frakes and Dillin, 2017). The UPR of the ER has been studied extensively and is highly conserved from fungi to mammalian cells. The accumulation of

misfolded proteins in the ER is sensed by three transmembrane signaling proteins, IRE-1, PERK and ATF6, which constitute distinct arms of the UPR and function to activate transcription factors for the production of a multiplicity of proteostasis components. PERK activation also leads to phosphorylation of eIF2α and thus to attenuation of general translation (Walter and Ron, 2011; Frakes and Dillin, 2017). Concomitantly, proteasome biogenesis is up-regulated by a recently discovered signaling pathway that adjusts cellular degradation capacity to demand (Rousseau and Bertolotti, 2016). Although the exact mode of activation of the UPR is distinct from that of the cytosolic heat-shock response, the overall goals are similar: an up-regulation of quality-control components and a decrease in potentially misfolded substrates though transient attenuation of translation.

Increasing evidence supports the existence of significant crosstalk between the various cellular stress-response pathways, with protein misfolding in the ER resulting in the aggregation of metastable proteins in the cytosol (Hamdan et al., 2017). This is consistent with ER stress triggering a partially protective cytosolic stress response when components of the UPR are defective (Liu and Chang, 2008). A link between mitochondrial stress and the cytosolic stress-response pathway has also been identified that can protect cells from disease associated aggregates (Kim et al., 2016a).

Chronic stress response

Although the up-regulation of protein quality-control components allows cells to resolve stress-induced misfolded proteins and aggregates that formed as the result of acute environmental stress, the amyloid aggregates associated with age-dependent diseases appear to be largely resistant to these rescue mechanisms (Klaips et al., 2014; Zaarur et al., 2015). The resulting chronic exposure of cells to misfolded species can have detrimental effects on the PN. For example, expression of model polyglutamine aggregates interferes with ER-associated protein degradation and leads to a prolonged activation of the ER stress response (Duennwald and Lindquist, 2008; Leitman et al., 2013).

On chronic production of certain misfolded or aggregated proteins, as may occur in disease or during aging, the stress response becomes activated but unable to clear the offending species (Lamech and Haynes, 2015; Fig. 4). This maladaptive stress response leaves cells vulnerable not only because the aggregates persist but also because the cells become refractive to additional stressors (Roth et al., 2014), consistent with aged cells and organisms being less responsive to stress insults (Ben-Zvi et al., 2009). A recent study in nematodes and mammalian cells revealed an interesting relationship among aging, chronic protein folding stress, and PN capacity (Tawo et al., 2017). These authors observed that normal turnover of the insulin-like growth factor receptor (Daf2 in *C. elegans*) involves the E3 ubiquitin ligase Chip. Both aging and the accumulation of protein aggregates were found to interfere with the degradation of insulin receptor, because Chip becomes increasingly engaged by misfolded proteins (Tawo et al., 2017). The resulting increase in Daf2 levels inhibits the Daf16 transcription factor (FOXO in mammals), causing a down-regulation of PN components and reduced lifespan.

Modulation of the PN

The persistence of disease-associated protein aggregates would suggest that the cellular PN is generally unable to cope with such substrates. However, cells may be able to adequately handle aberrant protein species for long periods, sometimes decades, as suggested by the fact that even the inherited forms of neurodegenerative disease, such as Huntington's disease, do not present until advanced age.

Indeed, specific modulation of PN components can impact both aggregate morphology and lifespan in model systems, paving the way for therapeutic intervention (Balch et al., 2008; Powers et al., 2009). Expression of chaperones and cochaperones of different classes have consistently resulted in a decrease in disease-aggregate toxicity and even increased lifespan (Auluck et al., 2002; Hoshino et al., 2011; Chafekar et al., 2012). Analogous to the cellular stress responses, strategies for therapeutic interventions in aggregate-associated neurodegenerative diseases have focused on preventing further production of misfolded species, stabilization of properly folded species, and clearance of existing aggregates (Balch et al., 2008; Calamini et al., 2011). Toward these ends, small molecules have been identified that prolong translation attenuation on stress (Tsaytler et al., 2011); stabilize mutant proteins, such as transthyretin against aggregation; target folding and trafficking defects in specific disease associated proteins, such as mutant cystic fibrosis transmembrane conductance regulator (Baranczak and Kelly, 2016); and increase the clearance of toxic protein species through activation of the UPS (Lee et al., 2010) or autophagy (Sarkar et al., 2009). Because of the broad range of components sequestered by protein aggregates, enhancement of the endogenous stress-response pathways themselves has been particularly fruitful in extending lifespan and proteostatic health (Sittler et al., 2001; Mu et al., 2008; Akerfelt et al., 2010; Kumsta et al., 2017).

Concluding remarks

Work over the past decades has uncovered a remarkable ability of cells to maintain proteostasis under a variety of challenging conditions. The importance of this ability is underlined by our increasing understanding that many neurodegenerative and aging-associated diseases are caused by a breakdown in this process. In recent years we have gained considerable insight as to why PN capacity may decline with age. Although in some cases the buildup of stochastic mutations and damage can certainly contribute to disease onset, this seems insufficient to explain the universal age-dependent decline in PN health observed across species. Work in metazoans such as worms suggests that this decline may instead be a regulated process. Consistent with the basic tenet of the "disposable soma" theory (Kirkwood and Holliday, 1979), organisms may sacrifice their own proteostatic fitness to divert resources toward reproduction. This is a plausible explanation, especially for short-lived species such as *C. elegans*, in which proteostasis decline occurs abruptly in early adulthood, and the lifespan extension gained by activation of stress-response factors comes at the cost of reduced fecundity (Ben-Zvi et al., 2009). However, the mechanisms underlying the gradual deterioration of proteostasis in long-lived mammals are clearly more complex. Hopefully, a better understanding of the network connectivity in healthy cells and tissues and its changes during aging and disease will allow us to harness aspects of the PN to combat aggregation disorders and increase health span.

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