Functional Modules of the Proteostasis Network

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Cells invest in an extensive network of factors to maintain protein homeostasis (proteostasis) and prevent the accumulation of potentially toxic protein aggregates. This proteostasis network (PN) comprises the machineries for the biogenesis, folding, conformational maintenance, and degradation of proteins with molecular chaperones as central coordinators. Here, we review recent progress in understanding the modular architecture of the PN in mammalian cells and how it is modified during cell differentiation. We discuss the capacity and limitations of the PN in maintaining proteome integrity in the face of proteotoxic stresses, such as aggregate formation in neurodegenerative diseases. Finally, we outline various pharmacological interventions to ameliorate proteostasis imbalance.

Proteins are the most versatile macromolecules and responsible for almost all cellular functions. An average human cell expresses ∼10,000–13,000 different protein species (Bekker-Jensen et al. 2017; Kulak et al. 2017) with copy numbers varying over several orders of magnitude, from a few molecules to tens of thousands. To maintain protein homeostasis, or proteostasis (Balchin et al. 2016), cells must ensure that these proteins fold and assemble correctly and exist in the right cellular locale at appropriate abundance.

Most proteins must adopt a unique, thermodynamically stable three-dimensional structure, the functionally active state, which is determined by the amino acid sequence (Anfinsen 1973). Proteins must fold to this native state at biologically relevant timescales, a process that is increasingly well understood, at least for small model proteins that fold rapidly without detectable intermediate states (Dobson and Karplus 1999; Hartl and Hayer-Hartl 2009). However, proteins with complex domain folds and multi-domain proteins, which make up the major part of the proteome, frequently populate folding intermediates that expose hydrophobic amino acid residues. These proteins are at risk of misfolding and aggregation within the highly crowded environment of the cell (Ellis and Minton 2006). Once folded, proteins must maintain their correct three-dimensional shape, which is complicated by the fact that functionality often requires considerable conformational flexibility. As a result, the folded states of many proteins are only marginally stable (or metastable) in the cellular milieu. Moreover, a considerable fraction of proteins (∼30% in human cells) are either completely devoid of ordered structure (intrinsically disordered proteins) or contain substantial unstructured regions (Dunker et al. 2008). These proteins typically only adopt folded structures dependent on binding to partner proteins,

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which emphasizes the importance of correct concentration and localization. Even natively structured proteins are constantly conformationally challenged in the face of a variety of endogenous and exogenous stresses. Proteins that fail to adopt or maintain their folded structure, as well as proteins that have served their function, must be removed by degradation to prevent deleterious consequences.

To maintain protein homeostasis and proper proteome balance, cells use interconnected modules of factors collectively referred to as the proteostasis network (PN). This network is comprised of the cellular machineries for the biogenesis and degradation of proteins with molecular chaperones as central coordinators mediating protein folding and conformational maintenance. Although early studies in yeast have enabled a basic understanding of the eukaryotic PN (Albanèse et al. 2006; Finley et al. 2012), the PN in human cells is predicted to be far more complex (Klaips et al. 2018), owing to the diverse requirements of cell- and tissue-specific proteomes (Wilhelm et al. 2014; Uhlen et al. 2015).

Loss of proteostasis is linked to aging and several medical conditions associated with the formation of toxic protein aggregates, prominently including numerous neurodegenerative diseases like Alzheimer's and Huntington's disease and other pathologies affecting many different cell types. The familial forms of these diseases have an underlying genetic cause in which mutations increase the likelihood of specific disease proteins to misfold and aggregate. Classic examples are forms of amyotrophic lateral sclerosis associated with mutations in superoxide dismutase and a group of disorders, including Huntington's disease, that are caused by expanded polyglutamine tracts in otherwise unrelated proteins. Importantly, most of these pathologies manifest in an age-dependent manner (age-of-onset diseases), consistent with a decline in proteostasis capacity during aging being a critical risk factor (Taylor and Dillin 2011; Labbadia and Morimoto 2015; Higuchi-Sanabria et al. 2018). Moreover, despite the presence of the respective mutation in all somatic tissues in most cases, specific cell types and tissues are exclusively or preferentially affected (Fu et al. 2018), suggesting an underlying heterogeneity between cell types in proteostasis capacity and the ability to respond to proteotoxic stress (Sala et al. 2017).

In this review, we discuss recent progress in our understanding of the modular organization of the PN and its capacity to maintain proteome integrity in the face of stress conditions. Using the example of stem cells, we examine PN plasticity and rewiring during cell differentiation. We further discuss how the burden of toxic protein aggregates in disease may cause proteostasis collapse. Finally, we outline various pharmacological interventions to ameliorate proteostasis imbalance as an attractive avenue toward therapeutic strategies for some proteinopathies.

ORGANIZATION OF THE PROTEOSTASIS NETWORK

Protein-folding accuracy is fundamental to all cells. However, maintaining fidelity of protein synthesis and folding is confounded by the fact that the underlying genetic and biochemical systems are inherently error prone, which leads to the constant production of a certain amount of misfolded proteins. This problem is further aggravated by genetic variation (single-nucleotide polymorphisms) and the effects of environmental stress. The resulting aberrant proteins must be detected and degraded by the PN to prevent them from engaging in unwanted interactions and toxic protein aggregation. Chronic aggregate production can overwhelm the cellular capacity to maintain proteostasis and respond properly to proteotoxic stress (Olzscha et al. 2011; Roth et al. 2014). To maintain a balanced and healthy proteome, cells have evolved an integrated network of protein quality-control factors (the PN) tending to the synthesis, folding, maintenance, and timely turnover of proteins. For any given protein, the PN comprises all cellular factors required for folding of the newly synthesized protein and conformational maintenance at the correct location and appropriate concentration, and finally for controlled degradation once the protein has fulfilled its function or has undergone irreversible misfolding.

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In its simplest form, the PN can be described by three major modules that govern each of these processes (Fig. 1A,B): (1) protein synthesis, (2) folding and conformational maintenance by molecular chaperones and cochaperones, and (3) protein degradation by the ubiquitin–proteasome system (UPS) and the autophagy–lysosomal pathway (ALP). These three modular branches coordinately tune the health of the proteome with molecular chaperones being central to their interconnectivity. Besides PN components that are required generally, such as ribosomes, proteasomes, and lysosomes, there is likely a requirement for "specific" factors tailored to certain proteins (or sets of proteins) with distinct properties. Additionally, proteostasis within organelles such as the endoplasmic reticulum (ER) and mitochondria requires specialized factors and chaperones for protein trafficking and folding (Hetz et al. 2015; Moehle et al. 2018). Because of this inherent complexity, the composition of the PN remains poorly defined. However, advances in understanding the functional annotation of components belonging to each individual module allows an estimation of a total size of ~2000 components in human cells (Fig. 1A; Klaips et al. 2018).

PROTEIN SYNTHESIS

Cytosolic ribosomes and associated factors produce the bulk of the cellular proteome at an average rate of five to six amino acids per second (Ingolia et al. 2011), generating more than a billion protein molecules per human cell (Milo 2013) with an average size of 560 amino acids (Wolff et al. 2014; Balchin et al. 2016). However, this process is error-prone with a misincorporation rate of 1 in 10^4 amino acids (Zaher and Green 2009). As a result, roughly 1 in 20 protein molecules contain a sequence error, potentially causing misfolding or reduced stability. Defective protein products can also arise as a result of posttranscriptional splicing errors or production of defective messenger RNA (mRNA). For example, erroneous mRNA molecules that lack stop codons cause ribosome stalling during translation. In these cases, the defective mRNAs are removed by cellular RNA surveillance path-ways (Doma and Parker 2007; Isken and Maquat 2007), and the translated nascent protein chain is degraded by the ribosome-associated quality-control (RQC) pathway (Brandman and Hegde 2016; Joazeiro 2017), which senses ribosome-stalled polypeptides and engages UPS components to degrade them. Failure of the RQC machinery leads to protein aggregation and proteotoxic stress (Choe et al. 2016; Yonashiro et al. 2016; Izawa et al. 2017) and age-dependent neurodegeneration in a mouse model (Chu et al. 2009). Transfer RNA (tRNA) availability may limit translation rates and result in protein aggregation, perhaps by increasing the likelihood of misreading or frameshifts, or increasing the risk that nascent chains occupy nonproductive folding intermediates (Nedialkova and Leidel 2015). Notably, the rate of protein synthesis is also dynamically regulated in response to a variety of stimuli, both globally and in a protein-specific manner. During conditions of proteotoxic stress, a programmed decrease in protein synthesis (Spriggs et al. 2010) is initiated (Figs. 1B and 2) to alleviate the load of newly synthesized proteins that must be folded by the PN. During conformational stress in the ER or activation of the integrated stress response, this is accomplished by phosphorylation of the translation initiation factor eIF2α (Pakos-Zebrucka et al. 2016; Karagöz et al. 2019).

FOLDING AND CONFORMATIONAL MAINTENANCE

As shown recently, some small proteins can undergo partial or complete folding within the exit tunnel of the ribosome (Holtkamp et al. 2015; Nilsson et al. 2015). However, for the majority of larger proteins, folding is thought to begin once a chain segment sufficient to specify a cooperatively folding domain has emerged from the ribosome, followed by completion of folding after chain release (Balchin et al. 2016). Multidomain proteins may fold their domains cotranslationally in a sequential manner, thereby reducing the problem of folding a large protein to the folding of smaller modules (Kim et al. 2013).

Both co- and posttranslational folding is assisted by molecular chaperones (Fig. 1B), which

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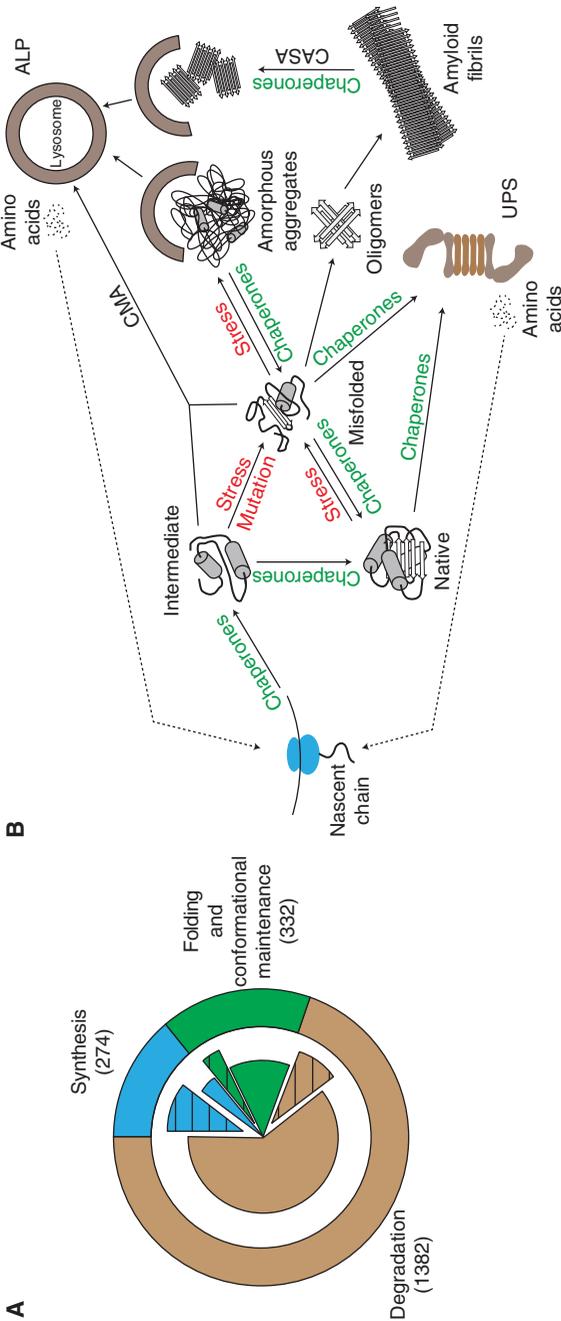
Figure 1. Architecture of the proteostasis network (PN). (*A*) The three modules of the PN (outer ring): synthesis (blue, 274 factors), folding and conformational maintenance (green, 332 factors), and degradation (brown, 1382 factors). The inner pie chart shows the partitioning of each module into essential (hatched sectors, synthesis [203/274], folding [78/332], and degradation [178/1382]) and nonessential components (filled sectors). (*B*) Central role of molecular chaperones in the PN. The nascent polypeptide chain emerging from the ribosomal tunnel is prevented from misfolding and aggregation by chaperones, including components that bind to the ribosome (trigger factor in bacteria, nascent chain–associated complex [NAC] and ribosome-associated complex [RAC] in eukarya). A second tier of chaperones (Hsp70, Hsp90, chaperonins) does not interact with the ribosome directly and mediates co- or posttranslational folding. Proteins that misfold because of mutations or under conditions of stress are selectively degraded either by the ubiquitin–proteasome system (UPS) or chaperone-mediated autophagy (CMA) and chaperone-assisted selective autophagy (CASA). Misfolded proteins may aggregate to soluble oligomers, amorphous aggregates, or amyloid fibrils when basal chaperone and degradation capacity is exceeded. Aggregates may be sequestered into insoluble inclusions, which may be dissociated into fragments by special-ized chaperones (Hsp104 in yeast, Hsp70/Hsp40/Hsp110 in metazoans) for subsequent clearance by the autophagy–lysosomal pathway (ALP).

prevent or reverse misfolding events and favor productive folding pathways. Molecular chaperones are defined as factors that assist in the folding (and often assembly) of another protein without being part of its final structure (Hartl 1996).

The human genome encodes ~332 chaperones and cochaperones (referred to as the "chaperome") (Fig. 1A; Brehme et al. 2014). Chaperones are broadly grouped by molecular weight and many are classified as heat shock proteins (Hsps) because their induction depends on exposure of cells to elevated temperature and other forms of proteome stress. The main classes of chaperones (88 proteins) include the ATP-dependent Hsp70s, Hsp90s, Hsp60s (also called chaperonins), and Hsp100s, and the ATP-independent small HSPs (sHsps). They are assisted by regulatory cochaperones (244 proteins) that further impart substrate specificity and selectivity. The major groups of cochaperones are the Hsp40s (49 proteins) as regulators of the Hsp70s, and the tetratricopeptide repeat proteins (TPR) (114) as regulators of the Hsp90 system (Table 1).

Chaperones serve as the main conductors of proteostasis by directly influencing the "foldedness" of the proteome. They typically do so by binding to hydrophobic polypeptide segments that are exposed by nonnative proteins but are buried in the native state, thus, preventing aggregation and maintaining the polypeptide in a

folding-competent state. The ATP-dependent chaperones (Hsp70, Hsp90, chaperonins) promote folding through ATP-regulated cycles of binding and release during which the client protein reaches its final structure, sometimes through an accelerated folding pathway (Hartl et al. 2011; Balchin et al. 2016). In some cases, proteins are altogether unable to fold without the aid of chaperones, defining the limit of the Anfinsen dogma. One such case is the cytoskeletal protein actin (Balchin et al. 2018).

Chaperones act in the folding of newly synthesized polypeptides either in association with the ribosome (e.g., the mammalian ribosome-associated complex [RAC] and specialized Hsp70s [HSP70L1]; Preissler and Deuerling 2012; Kramer et al. 2018) or downstream from the ribosome after polypeptide release, such as the classical Hsp70s, Hsp90s, and the TRiC/ CCT chaperonin (Fig. 1B). TRiC (and the chaperonins in mitochondria, Hsp60, and bacteria, GroEL) are large ~1 MDa double-ring complexes that transiently enclose a single molecule of unfolded substrate protein in a cage-like structure, allowing folding to occur unimpaired by aggregation (Balchin et al. 2016; Hayer-Hartl et al. 2016). The ATP-independent sHsps function as "holdases" in stabilizing proteins against aggregation or in shielding the surfaces of aggregates (Carra et al. 2017).

Molecular chaperones cooperate in cellular folding pathways as shown for the bacterial and eukaryotic cytosol and in other systems (Langer et al. 1992; Frydman et al. 1994; Frydman 2001). Indeed, different chaperones interact to form modules and functional supercomplexes (Rizzolo et al. 2017). Specific chaperone pathways for the diverse array of protein clients can be perceived as a sequential percolator. Proteins that fold rapidly after interaction with upstream chaperones, like the Hsp70s, would not require more specialized downstream chaperones, whereas proteins that fail to fold with the upstream chaperones will filter down to Hsp90 or the chaperonin to attain their folded functional state (Hartl 1996).

Quantitative analyses of client proteins for specific chaperones in simpler model systems like *Escherichia coli* (Kerner et al. 2005; Oh

Table 1. Chaperone classification in human cells

Chaperone class	Number of genes
HSP40	49
HSP60	15
HSP70	27
HSP90	46
HSP100	14
sHSP	10
TPR	114
Others (prefoldin, calnexin, oxidoreductases, prohibitin)	57

Data adapted from Brehme et al. (2014).
HSP, Heat shock protein; sHSP, small heat shock protein; TPR, tetratricopeptide repeat protein.

et al. 2011; Calloni et al. 2012) have revealed features of client flux through the bacterial chaperone network. Similar studies in *Saccharomyces cerevisiae* have yielded a systems view of the eukaryotic chaperone network (Zhao et al. 2005; Gong et al. 2009; Willmund et al. 2013). Chaperone-dependent proteins are frequently larger than average, containing multiple domains or domains with complex fold topologies that are typically characterized by many long-range interactions. Salient features of the eukaryotic chaperone network have emerged from systematic mapping of the interactions of chaperones and cochaperones with their clients. It has been shown for yeast cells that the "chaperome" can be divided into components that are mainly devoted to the folding of newly synthesized proteins and those that function in conformational maintenance (Albanèse et al. 2006). The former includes the ribosome-binding chaperones that are not stress inducible, whereas the latter include the classic stress-inducible chaperones.

In humans and other mammals, the chaperome has expanded to match the needs of a larger and more complex proteome (Powers and Balch 2013). Although the PN of human cells has not yet been comprehensively characterized, recent studies focused on the substrate interactome of Hsp90, a chaperone system thought to be specifically devoted to kinases and other signaling proteins. In an initial survey of ∼2000 candidates comprising kinases, transcription factors (TFs) and E3 ligases, ∼200 of 355 kinases and ∼120 of 426 ubiquitin E3 ligases analyzed were found to use Hsp90 for folding or conformational regulation, whereas TFs showed no such preference (50 Hsp90 substrates among 1100 TFs tested) (Taipale et al. 2012). The finding that E3 ligases are clients of the folding machinery further emphasizes the interconnectivity of the folding and degradation modules of the PN. A more comprehensive investigation (Taipale et al. 2014), including ∼70 chaperones, cochaperones, and protein quality-control factors, revealed a hierarchical organization of the chaperone network centered on the interconnected Hsp70 and Hsp90 chaperone systems. More recently, an integrated chaperone network that supports cell survival was observed in cancer cells to be essential for tumor maintenance. This complex called the "epichaperome" (Rodina et al. 2016) is formed by an enhanced physical interaction of the Hsp70 and Hsp90 machineries along with their cochaperones.

A key question regarding chaperone networks is how client flux is redirected when one chaperone system is overwhelmed. Similar to findings in bacterial cells in which mutual compensation among chaperone systems is observed (Deuerling et al. 1999; Teter et al. 1999; Calloni et al. 2012), inhibition of Hsp90 in human cells promoted client binding to Hsc70, the constitutively expressed Hsp70 (Taipale et al. 2014). Among the BAG family cochaperones, which function as nucleotide exchange factors of Hsp70, only BAG2 proved to have a client range similar to Hsc70, suggesting that it is a general cofactor involved in the folding pathway of Hsc70 substrate proteins. The interaction spectrum of Hsc70 also correlates with that of the E3 ligase CHIP (carboxy terminus of Hsp70-interacting protein), again pointing to a functional cooperation between chaperones and the UPS (Esser et al. 2004; Kundrat and Regan 2010). Although these studies have provided initial insights into how substrate proteins are distributed within the chaperome, it remains to be established how exactly client specificity and selectivity is achieved. Within the Hsp70 system, the Hsp40 cofactors (∼50 different proteins in human) play a central role in this process (Table 1; Kampinga and Craig 2010).

Although the majority of chaperone systems function to prevent protein aggregation, certain chaperones are specialized to act as "disaggregases" and actively dissolve protein aggregates (Fig. 1B; Mogk et al. 2018). A well-studied example is yeast Hsp104, which performs disaggregation in an ATP-driven manner (Parsell et al. 1994) in cooperation with Hsp70 and Hsp40 (Mogk et al. 2015). In metazoans, which lack Hsp104, disaggregation of stress-induced aggregates is enabled by Hsp70 in cooperation with Hsp110 (an Hsp70-like protein that functions as nucleotide exchange factor for Hsp70), Hsp40s, and sHsps (Nillegoda et al. 2015; Żwirowski et al. 2017).

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PROTEIN DEGRADATION

The steady-state concentration of proteins is the result of a balance between protein synthesis and degradation. Cells are equipped with efficient protein degradation machineries that recycle proteins once they are no longer needed, and remove misfolded proteins to prevent the formation of potentially toxic aggregates. Eukaryotic cells use two main degradation machineries, the UPS and the ALP (Förster et al. 2013; Cohen-Kaplan et al. 2016; Dikic 2017; Varshavsky 2017). The UPS serves as the major pathway of protein degradation and is responsible for ~80% of protein turnover (Zhao et al. 2015; Collins and Goldberg 2017). Central to the UPS is the 26S proteasome, a large multisubunit protease complex (Förster et al. 2013; Livneh et al. 2016). The 26S proteasome recognizes substrates after their covalent modification with the small protein ubiquitin. Ubiquitylation of target proteins is a highly regulated process that is catalyzed in an ATP-dependent manner by a cascade of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3; Finley 2009; Zheng and Shabek 2017). Of the ~2000 PN components, the UPS constitutes the largest group with ~850 proteins (Klaips et al. 2018), ~600 of which are E3 ligases (Fig. 1A; Li et al. 2008). The catalytic sites of the proteasome are buried within the central cavity of the 20S catalytic unit, and can only be accessed if the protein substrate is able to pass a narrow entrance into the catalytic core. To achieve this passage, most proteins need to be unfolded in an ATP-dependent reaction catalyzed by the 19S regulatory particle that caps the 20S core (Bhattacharyya et al. 2014). Recent cryoelectron microscopy analyses of the proteasome with substrate protein engaged provide mechanistic insight into this complex process (Beck et al. 2012; de la Peña et al. 2018; Dong et al. 2019). Owing to the requirement for substrate unfolding, the proteasome is unable to degrade aggregated proteins directly, and disaggregation by chaperone machinery is a prerequisite. More generally, chaperones function to stabilize misfolded proteins in a nonaggregated state competent for ubiquitin tagging and delivery to the proteasome (Esser et al. 2004). A chaperone-bound protein may be triaged for degradation by ubiquitylation when (re)folding is unsuccessful, resulting in a prolonged chaperone residence time. The E3 ligase CHIP interacts directly with Hsp70 to ubiquitylate chaperone-bound proteins. Up to a certain point, this modification may still be reversed by ubiquitin hydrolases (Mevissen and Komander 2017). How these opposing reactions are fine-tuned is central to understanding the mechanisms of protein quality control.

Despite the presence of the UPS, protein misfolding nevertheless leads to the formation of insoluble aggregates and larger inclusions, especially under stress conditions. In contrast to the UPS, autophagy coupled to lysosomal degradation has the capacity to directly remove such aggregates (Fig. 1B). In this process, aggregates or fragments thereof are engulfed by double-membrane structures (autophagosomes), which then fuse with the lysosome (Galluzzi et al. 2017). Although the core machinery of autophagy consists of only 17 proteins (Dikic 2017), the ALP system, including accessory factors, comprises a total of ~500 components (García-Prat et al. 2016). Aggregated proteins accumulate in ubiquitin-positive deposits to which the autophagic machinery is recruited by chaperones in a process known as chaperone-assisted selective autophagy (CASA) (Carra et al. 2008; Gamerdinger et al. 2009; Arndt et al. 2010). Under basal conditions in mammalian cells, soluble proteins can also be degraded by a specific form of autophagy called chaperone-mediated autophagy (CMA), which involves substrate recognition and unfolding by Hsc70 and direct lysosomal translocation by binding to the lysosomal receptor LAMP2A, without the need for autophagosome formation (Fig. 1B; Kaushik and Cuervo 2018). Whereas both the UPS and ALP display a certain degree of specificity regarding their range and diversity of substrates, they are functionally interconnected and often compensate one another under conditions in which one pathway is saturated or malfunctional (Suraweera et al. 2012; Zhang et al. 2016; Dikic 2017)—another reflection of the network character of the PN.

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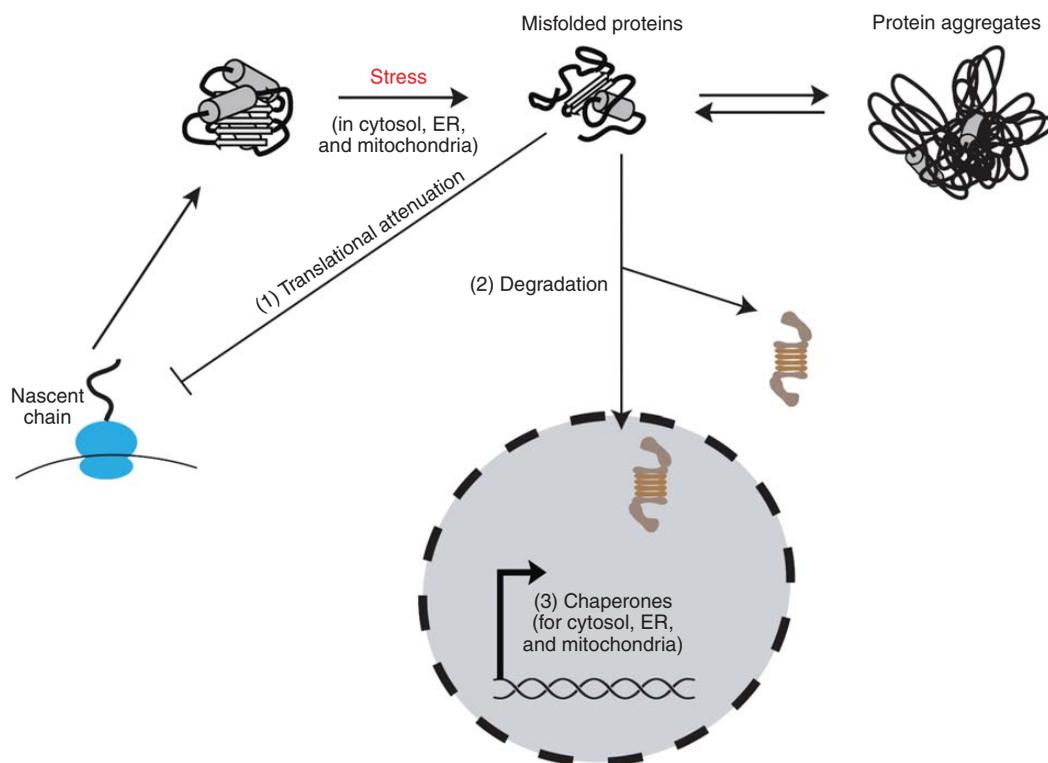
Figure 2. Proteostasis capacity and stress responses. During proteotoxic stress in different cellular compartments, proteostasis capacity is adjusted to meet increased cellular requirements. Global translational attenuation (*1*) reduces the burden on the folding machineries to free chaperone capacities for assistance in clearance of misfolded and aggregated species (*2*). Concomitantly, transcriptional stress-response pathways are activated (*3*) to increase chaperone pools available for folding and degradation. ER, Endoplasmic reticulum.

STRESS-RESPONSE PATHWAYS

Many natively folded proteins are metastable under normal cellular growth conditions (i.e., they reside at the limit of thermodynamic stability or contain substantial intrinsically disordered regions). Exposing cells to proteome stress, such as heat stress, then results in (partial) protein unfolding and possible aggregation. To deal with such stress situations, cells have evolved compartment-specific transcriptional stress-response pathways (Fig. 2), prominently including the cytosolic heat stress response (HSR) (Morimoto 2011) and unfolded protein response pathways of the ER (UPR^{ER}) (Walter and Ron 2011; Karagöz et al. 2019) and mitochondria (UPR^{Mito}) (Melber and Haynes 2018; Uma Naresh and Haynes 2019).

Activation of the HSR leads to the induction of molecular chaperones (Hsps) and other quality-control components, which act to prevent protein aggregation and mediate protein refolding or degradation via the UPS (Richter et al. 2010). The selective induction of molecular chaperones is accompanied by a global decrease in protein synthesis, particularly through attenuation of translational elongation (Liu et al. 2013; Shalgi et al. 2013), thus reducing the burden on the folding machinery and making proteostasis capacity available for conformational maintenance and clearance of misfolded proteins (Fig. 2). Induction of the HSR is not limited to acute abiotic stresses, such as heat or a change in pH, but is also mediated by other proteotoxic insults, such as the expression of certain misfolding mutant proteins at normal cellular

growth temperatures (Geiler-Samerotte et al. 2011).

The eukaryotic HSR is mediated by heat shock factor 1 (HSF1) as the master TF (Vabulas et al. 2010; Anckar and Sistonen 2011). Under normal conditions, HSF1 exists as a monomer bound by chaperones (Zou et al. 1998; Neef et al. 2014; Zheng et al. 2016). Although HSF1 controls the expression of thousands of genes, its key function under conditions of proteotoxic stress is the tight regulation of a small set of chaperone genes (Mahat et al. 2016; Solís et al. 2016). The current model of the HSR posits that production of misfolded proteins under conditions of stress titrates away the chaperones bound to HSF1, which allows HSF1 to trimerize and become transcriptionally active. The resulting increase in chaperone levels provides a negative feedback loop that deactivates HSF1 once a sufficient amount of free chaperones is restored (Krakowiak et al. 2018). Additional mechanisms of attenuation of the HSR include HSF1 degradation and cycles of acetylation and deacetylation (Westerheide et al. 2009; Raychaudhuri et al. 2014).

Although it is unclear what fraction of the mammalian proteome denatures and misfolds during heat stress (Wallace et al. 2015), the level of ubiquitylated proteins rapidly increases in stressed cells (Fujimuro et al. 1997), suggesting that degradation of misfolded proteins via the UPS is critical in reestablishing proteome balance (Parag et al. 1987). Nascent polypeptides synthesized prior to heat stress, possibly still bound to ribosomes in unfolded states, are thought to represent the major vulnerable fraction of the proteome that is targeted for degradation (Medicherla and Goldberg 2008; Xu et al. 2016). A comprehensive genome-wide deletion screen for induction of the HSR in yeast (Brandman et al. 2012) under nonstress conditions revealed an enrichment of individual chaperones and members of the UPS, among other inducers, pointing to a strong cross talk between the two PN modules. Future genome-wide efforts to study proteostasis in mammalian cells will be facilitated by the advent of pooled CRISPR screens (Shalem et al. 2014) and haploid genetic screens (Brockmann et al. 2017).

Although protein degradation via the UPS has been classically associated with the HSR, studies in the nematode *Caenorhabditis elegans* (Kumsta et al. 2017) and mammalian cells (Watanabe et al. 2017) showed a direct link between the HSR and autophagy, thus providing further evidence for a highly interconnected PN with modular flexibility.

THE PROTEOSTASIS NETWORK IN CELL DIFFERENTIATION

All existing tissues and cell types within the human body are a result of well-defined programs of stem cell division and differentiation. Although embryonic stem cells (ESCs) represent the reservoir of pluripotent cells and thus shape development early in life, postnatal maintenance of tissue homeostasis is conducted by specialized multipotent adult stem cells (e.g., neuronal stem cells and satellite stem cells in muscles) within their respective niches. Regardless of their origins, stem cells must continuously replenish their numbers via asymmetric cell division (segregating cell fate determinants into only one of the two daughter cells) (Knoblich 2008), while maintaining a damage-free proteome. At the same time, they have to remodel their proteomes as they differentiate into various fates, which emphasize the importance of proteostasis control and PN rewiring in development (Vilchez et al. 2014).

Stem cells are thought to have a higher proteostasis capacity than differentiated cells. Lower rates of protein synthesis in adult hematopoietic, neural, epidermal, and muscle stem cells (Signer et al. 2014; Llorens-Bobadilla et al. 2015; Blanco et al. 2016; Zismanov et al. 2016) presumably result in a reduced burden on the folding module of the PN. In contrast, ESCs tend to have higher translational rates (Ingolia et al. 2011; You et al. 2015). This difference can be attributed to the higher proliferative activity of ESCs (García-Prat et al. 2017). Important differences have also been noted in the composition of the stem cell chaperome (Fig. 3). ESCs have higher levels of a subset of chaperones, such as the chaperonin TRiC/CCT, which decreases as cells differentiate into neuronal progenitors and

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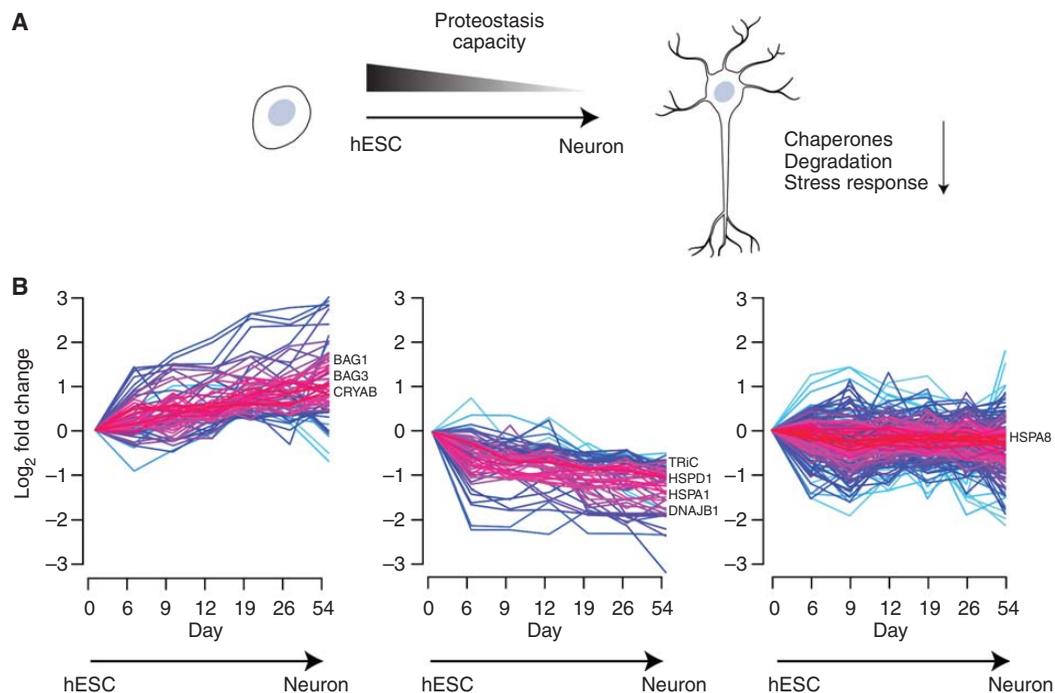
Figure 3. Proteostasis network (PN) rewiring and decline in proteostasis capacity during cell differentiation. (*A*) Human embryonic stem cells (hESCs) have a higher proteostasis capacity as compared to differentiated cells, such as neurons. As hESCs differentiate to neurons, the chaperonins (cytosolic TRiC/CCT and mitochondrial Hsp60) decrease in abundance and there is a general decline in degradation capacity. Additionally, differentiated neurons are less efficient in mounting a stress response in comparison to their progenitors and stem cells. (*B*) Chaperome trajectories during neuronal differentiation of hESCs (332 proteins analyzed). The chaperome landscape changes in abundance (indicated as log$_2$ fold change) as hESCs are differentiated into neurons. Chaperones can increase (*left*), decrease (*middle*), or remain unchanged in abundance (*right*) during this process. Prominent examples of each cluster are shown (BAG1/BAG3, regulators of Hsp70/Hsc70; CRYAB, α-crystallin B chain; TRiC, cytosolic chaperonin; HSPD1, mitochondrial Hsp60; HSPA1, Hsp70; DNAJB1, Hsp40 regulator of Hsp70/Hsc70; HSPA8, Hsc70). Data for time-dependent changes in chaperone abundance values were derived from Sequence Read Archive (SRA) accession No. GSE86985 (Yao et al. 2017). TPM (transcripts per million) values were directly used to calculate clustering of chaperone trajectories using the fuzzy c-means algorithm Mfuzz (Kumar and Futschik 2007) with "*m*" values set at 2 and a minimum membership cutoff of 0.8. The gradient from red to blue indicates high to low membership scores.

neurons (Noormohammadi et al. 2016). The high demand for TRiC/CCT may be related to the folding requirements of actin (Yam et al. 2008; Balchin et al. 2018), which is increasingly needed in proliferating ESCs for cytoskeletal synthesis and integrity. Mouse ESCs have also been found to have increased levels of stress-inducible cytosolic Hsp70 (HSPA1), a strong target of HSF1 (Saretzki et al. 2004, 2008). Hsp70 levels decrease as the cells differentiate, which may point to a dampened stress response.

Indeed, neural differentiation from progenitor cells results in a marked reduction of HSR inducibility (Fig. 3A; Yang et al. 2008), suggesting that neurons may inherently be more vulnerable to proteome stress. It remains to be seen whether this is generally true for other differentiated tissues.

Stem cells are also equipped with enhanced protein degradation capacity, presumably allowing for the effective removal of misfolded proteins. Studies in ESCs showed higher levels of

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proteasome activity associated with an increase of the 19S regulatory subunit PSMD11, which is limiting for 26S proteasome assembly (Vilchez et al. 2012). Proteasome activity decreased during differentiation into several lineages.

An extreme case of PN rewiring and proteome remodeling is the recently described mechanism of terminal differentiation of reticulocytes into erythrocytes (red blood cells) within the hematopoietic lineage (Nguyen et al. 2017). Erythrocytes are unique cells whose proteome is composed to almost 98% of a single protein—hemoglobin. As reticulocytes differentiate into erythrocytes, most of their proteome is degraded and replaced by hemoglobin. Surprisingly, the determinant of such a global process is a single E2-E3 hybrid ligase, UBE2O, which encodes activities for multiple components of a quality-control pathway—substrate recognition (in lieu of a chaperone), ubiquitin transfer (E2), and ubiquitin conjugation (E3). UBE2O was also found to be responsible for the removal of ribosomal proteins via the proteasome, a hallmark of erythrocyte development. UBE2O also clears unassembled globin chains, thus, serving as an essential quality-control factor during this transition (Yanagitani et al. 2017). This unique process exemplifies extensive rewiring of PN modules.

AMELIORATING PROTEINOPATHIES

PN deregulation and decline during aging has been recognized as a major driver of many proteinopathies, especially affecting the more vulnerable populations of postmitotic neuronal cells (Dillin and Cohen 2011; Gidalevitz et al. 2011; Morimoto 2019). These age-onset pathologies include Alzheimer's, Parkinson's, and Huntington's diseases, as well as amyotrophic lateral sclerosis and other pathologies. All these disorders are characterized by the formation of soluble, oligomeric, and insoluble fibrillar aggregates that exert cytotoxic effects (Chiti and Dobson 2017; Iadanza et al. 2018). Of particular importance in understanding the progressive nature of these diseases is that the aggregates subvert the function of the PN at several levels, for example, by sequestering critical chaperone

components and degradation machinery into aggregate inclusions (Rubinsztein 2006; Park et al. 2013; Yu et al. 2014; Guo et al. 2018; Thibaudeau et al. 2018), overburdening the UPS (Bence et al. 2001; Bennett et al. 2007; Hipp et al. 2012), and reducing the cellular capacity to respond to additional stresses (Labbadia et al. 2011; Olzscha et al. 2011; Roth et al. 2014). As a result, affected cells gradually lose the ability to cope with misfolding proteins, which in turn increases aggregate burden, setting in motion a vicious cycle that ends in proteostasis collapse and cell death (Hipp et al. 2014).

Because the PN is inherently modular and adaptable to stress, exploring ways to boost proteostasis could provide therapeutically viable options to decrease disease severity and slow progression (Balch et al. 2008; Powers et al. 2009; Hipp et al. 2014). Evidence has been provided that molecular chaperones, especially Hsp70 and Hsp40, can prevent formation of amyloid fibers (Muchowski et al. 2000) or disaggregate them (Gao et al. 2015), showing the potential of modulating the PN to combat proteinopathies in vivo. Accordingly, pharmacological induction or genetic overexpression of molecular chaperones has been shown to prevent aggregation in cellular (Sittler et al. 2001) and animal models (Labbadia et al. 2012; Kakkar et al. 2016; Nagy et al. 2016; Scior et al. 2018).

Although boosting chaperone activities has shown promise, tuning the other PN modules may also enhance proteostasis capacity. Small molecule-based modulation of the UPS (Lee et al. 2010; Leestemaker et al. 2017) and autophagy (Sarkar et al. 2009; Barmada et al. 2014; Kuo et al. 2015) augments clearance of protein aggregates and improves cellular health. Furthermore, stress responses to protein misfolding often culminate in translational attenuation to reduce downstream burden on the folding and degradation arm of the PN (Fig. 2). Reducing the level of newly synthesized proteins is therefore another promising approach to combat protein misfolding. Indeed, the pharmacologic prolongation of stress-induced translational attenuation has been shown to be beneficial in protein-misfolding disorders (Tsaytler et al. 2011; Das et al. 2015).

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CONCLUDING REMARKS

Maintenance of proteostasis is central to cellular health, and its impairment is causally linked to neurodegenerative proteinopathies and numerous other pathologies. Advances over the past two decades have laid the foundation for our understanding of the architecture of the PN. The modular and adaptive nature of the PN enables cells to handle changing proteomic requirements during cellular development and endure adverse conditions brought about by various endogenous and environmental stresses. Although we currently have only a bird's-eye view of how the PN is organized and how many components may be involved in proteostasis maintenance, future studies will hopefully tease apart the complex and specific PN connectivities in tissues that are particularly vulnerable to proteostasis collapse. Progress resulting from such studies will be especially relevant in developing urgently needed treatments for the group of neurodegenerative proteinopathies. Protein misfolding and aggregation in these diseases result in a gain-of-function toxicity, which eventually overwhelms the capacity of the PN to maintain proteostasis. Enhancing proteostasis capacity through modular tuning of the PN offers viable strategies for therapeutic intervention.

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