

Functional Modules of the Proteostasis Network

Gopal G. Jayaraj, Mark S. Hipp and F. Ulrich Hartl

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

Abstract

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.

Introduction

Proteins are the most versatile macromolecules and are responsible for almost all cellular functions. An average human cell expresses approximately 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, that 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; IDRs) or contain substantial unstructured regions (Dunker et al. 2008). These proteins typically only adopt folded structures upon binding to partner proteins, 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, for example due to mutation, as well as proteins which have served their function, must be removed by degradation to prevent deleterious consequences.

To maintain protein homeostasis and proper proteome balance, cells employ interconnected modules of factors collectively referred to as the proteostasis network (PN). This network comprises of 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 (Albanese 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 ageing 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, despite the presence of the respective mutation in all somatic tissues, most of these pathologies manifest in an age-dependent manner (age-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, 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 towards 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 upon synthesis 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.

In its simplest form the PN can be described by three major modules which govern each of these processes (Figure 1A and B): 1) protein synthesis; 2) folding and conformational maintenance by molecular chaperones and co-chaperones; 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). Due to 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 (Klaips et al. 2018) (Figure 1A).

Protein Synthesis

Cytosolic ribosomes and associated factors produce the bulk of the cellular proteome at an average rate of 5-6 amino acids per second (Ingolia et al. 2011), generating over 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 contains a sequence error, potentially causing misfolding or reduced stability. Defective protein products can also arise due to posttranscriptional splicing errors or production of defective 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 pathways (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 to age-dependent neurodegeneration in a mouse model (Chu et al. 2009). tRNA availability may limit translation rates and result in protein aggregation, perhaps by increasing the likelihood of misreading or frameshifts, or by increasing the risk that nascent chains occupy non-productive 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 (Figure 1B) to alleviate the load of newly-synthesized proteins that must be folded by the PN. Upon conformational stress in the ER or activation of the integrated stress response, this is accomplished by phosphorylation of the translation initiation factor eIF2 α (Pakos-Zebrucka et al. 2016; Karagöz et al. this vol.).

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 upon chain release (Balchin et al. 2016). Multi-domain proteins may fold their domains co-translationally 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 post-translational folding is assisted by molecular chaperones (Figure 1B), which prevent or reverse misfolding events and favor productive folding pathways. Molecular chaperones are defined as factors which 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 co-chaperones (referred to as the 'chaperome') (Brehme et al. 2014) (Figure 1A). Chaperones are broadly grouped by molecular weight and many are classified as heat shock proteins (Hsp), due to their induction upon 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 heat shock proteins (sHsps). They are assisted by regulatory co-chaperones (244 proteins) that further impart substrate specificity and selectivity. The major groups of co-chaperones 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 non-native 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 of the ribosome upon polypeptide release, such as the classical Hsp70s, Hsp90s and the TRiC/CCT chaperonin (Figure 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 upon 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 *E. coli* (Kerner et al. 2005; Oh et al. 2011; Calloni et al. 2012) have revealed features of client flux through the bacterial chaperone network. Similar studies in *S. 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, 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 co-chaperone 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 components that function in conformational maintenance (Albanese et al. 2006). The former include 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). While 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 (Mymrikov et al. 2017). 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 utilize Hsp90 for folding or conformational regulation, while 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, co-chaperones 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 co-chaperones.

A key question regarding chaperone networks is how client flux is redirected when one chaperone system is overwhelmed. Similar to findings in bacterial cells where 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 co-chaperones, 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 co-factor 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 co-operation between chaperones and the UPS (Esser et al. 2004; Kundrat and Regan 2010). While 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 (Kampinga and Craig 2010) (Table 1).

While the majority of chaperone systems function to prevent protein aggregation, certain chaperones are specialized to act as "disaggregases" and actively dissolve protein aggregates (Mogk et al. 2018) (Figure 1B). 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 (a Hsp70-like protein that functions as nucleotide exchange factor for Hsp70), Hsp40s and sHsps (Nillegoda et al. 2015; Zwirowski et al. 2017).

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 which recycle proteins once they are no longer needed and remove misfolded proteins in order to prevent the formation of potentially toxic aggregates. Eukaryotic cells employ two main degradation machineries, the UPS and the autophagy lysosomal pathway (ALP) (Forster 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 (Forster 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 (Li et al. 2008) (Figure 1A). 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 Pena 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 non-aggregated 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 (Mevisen 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 (Figure 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). While the core machinery of autophagy consists of only 17 proteins (Dikic 2017), the ALP system including accessory factors comprises a total of ~500 components (Garcia-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 (Kaushik and Cuervo 2018) (Figure 1B). While both the UPS and the 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 where 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.

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 IDRs). 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 (Figure 2), prominently including the cytosolic heat stress response (HSR) (Morimoto 2011) and the unfolded protein response pathways of the ER (UPR^{ER}) (Walter and Ron 2011; Karagöz et al. this vol.) and mitochondria (UPR^{Mito}) (Melber and Haynes 2018; Naresh and Haynes, this vol.).

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 (Figure 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 transcription factor (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). While 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; Solis 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).

While 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 non-stress 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). While protein degradation via the UPS has been classically associated with the HSR, studies in the nematode *C. elegans* (Kumsta et al. 2017) and in mammalian cells (Watanabe et al. 2017) demonstrated 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. While 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 multi-potent 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 emphasizes 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 (Garcia-Prat et al. 2017). Important differences have also been noted in the composition of the stem cell chaperome (Figure 3A and B). ESCs have higher levels of a subset of chaperones, such as the chaperonin TRiC/CCT, which decreases as cells differentiate into neuronal progenitors and 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; Saretzki et al. 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 (Yang et al. 2008) (Figure 3A), 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 demonstrated higher levels of 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, this vol.). 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; Thibautaud et al. 2018), by overburdening the UPS (Bence et al. 2001; Bennett et al. 2007; Hipp et al. 2012), and by 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).

Since 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), demonstrating 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).

While 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 (Figure 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).

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 proteostasis network. 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 results 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.

Table 1. Chaperone classification in human cells (from Brehme et al. 2014)

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

Figure legends

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 mediate co- or post-translational folding. Proteins that misfold due to mutations or under conditions of stress are selectively degraded either by the ubiquitin proteasome system (UPS) or by chaperone-mediated autophagy (CMA) and chaperone-mediated 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 specialized chaperones (Hsp104 in yeast, Hsp70/Hsp40/Hsp110 in metazoans) for subsequent clearance by the autophagy/lysosomal pathway (APL).

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 responses

pathways are activated (3) to increase chaperone pools available for folding and degradation.

Figure 3. Proteostasis network rewiring and decline in proteostasis capacity during cell differentiation.

A. Human embryonic stem cells (hESC) have a higher proteostasis capacity as compared to differentiated cells, such as neurons. As hESC 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 hESC (332 proteins analyzed). The chaperome landscape changes in abundance (indicated as \log_2 fold change) sampled on each day as hESC 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 number 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.

References

- Albanese V, Yam AY, Baughman J, Parnot C, Frydman J. 2006. Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. *Cell* **124**: 75-88.
- Anckar J, Sistonen L. 2011. Regulation of HSF1 function in the heat stress response: implications in aging and disease. *Annu Rev Biochem* **80**: 1089-1115.
- Anfinsen CB. 1973. Principles that govern the folding of protein chains. *Science* **181**: 223-230.
- Arndt V, Dick N, Tawo R, Dreiseidler M, Wenzel D, Hesse M, Furst DO, Saftig P, Saint R, Fleischmann BK et al. 2010. Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr Biol* **20**: 143-148.
- Balch WE, Morimoto RI, Dillin A, Kelly JW. 2008. Adapting proteostasis for disease intervention. *Science* **319**: 916-919.
- Balchin D, Hayer-Hartl M, Hartl FU. 2016. In vivo aspects of protein folding and quality control. *Science* **353**: aac4354.
- Balchin D, Milicic G, Strauss M, Hayer-Hartl M, Hartl FU. 2018. Pathway of actin folding directed by the eukaryotic chaperonin TRiC. *Cell* **174**: 1507-1521 e1516.
- Barmada SJ, Serio A, Arjun A, Bilican B, Daub A, Ando DM, Tsvetkov A, Pleiss M, Li X, Peisach D et al. 2014. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. *Nat Chem Biol* **10**: 677-685.
- Beck F, Unverdorben P, Bohn S, Schweitzer A, Pfeifer G, Sakata E, Nickell S, Plitzko JM, Villa E, Baumeister W et al. 2012. Near-atomic resolution structural model of the yeast 26S proteasome. *Proc Natl Acad Sci U S A* **109**: 14870-14875.
- Bekker-Jensen DB, Kelstrup CD, Batth TS, Larsen SC, Haldrup C, Bramsen JB, Sorensen KD, Hoyer S, Orntoft TF, Andersen CL et al. 2017. An optimized shotgun strategy for the rapid generation of comprehensive human proteomes. *Cell Syst* **4**: 587-599 e584.
- Bence NF, Sampat RM, Kopito RR. 2001. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* **292**: 1552-1555.
- Bennett EJ, Shaler TA, Woodman B, Ryu KY, Zaitseva TS, Becker CH, Bates GP, Schulman H, Kopito RR. 2007. Global changes to the ubiquitin system in Huntington's disease. *Nature* **448**: 704-708.
- Bhattacharyya S, Yu H, Mim C, Matouschek A. 2014. Regulated protein turnover: snapshots of the proteasome in action. *Nat Rev Mol Cell Biol* **15**: 122-133.
- Blanco S, Bandiera R, Popis M, Hussain S, Lombard P, Aleksic J, Sajini A, Tanna H, Cortes-Garrido R, Gkatza N et al. 2016. Stem cell function and stress response are controlled by protein synthesis. *Nature* **534**: 335-340.
- Brandman O, Hegde RS. 2016. Ribosome-associated protein quality control. *Nat Struct Mol Biol* **23**: 7-15.
- Brandman O, Stewart-Ornstein J, Wong D, Larson A, Williams CC, Li GW, Zhou S, King D, Shen PS, Weibezahn J et al. 2012. A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell* **151**: 1042-1054.
- Brehme M, Voisine C, Rolland T, Wachi S, Soper JH, Zhu Y, Orton K, Vilella A, Garza D, Vidal M et al. 2014. A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep* **9**: 1135-1150.
- Brockmann M, Blomen VA, Nieuwenhuis J, Stickel E, Raaben M, Bleijerveld OB, Altelaar AFM, Jae LT, Brummelkamp TR. 2017. Genetic wiring maps of single-cell protein states reveal an off-switch for GPCR signalling. *Nature* **546**: 307-311.

- Calloni G, Chen T, Schermann SM, Chang HC, Genevaux P, Agostini F, Tartaglia GG, Hayer-Hartl M, Hartl FU. 2012. DnaK functions as a central hub in the E. coli chaperone network. *Cell Rep* **1**: 251-264.
- Carra S, Alberti S, Arrigo PA, Benesch JL, Benjamin IJ, Boelens W, Bartelt-Kirbach B, Brundel B, Buchner J, Bukau B et al. 2017. The growing world of small heat shock proteins: from structure to functions. *Cell Stress Chaperones* **22**: 601-611.
- Carra S, Seguin SJ, Lambert H, Landry J. 2008. HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. *J Biol Chem* **283**: 1437-1444.
- Chiti F, Dobson CM. 2017. Protein misfolding, amyloid formation, and human disease: A summary of progress over the last decade. *Annu Rev Biochem* **86**: 27-68.
- Choe YJ, Park SH, Hassemer T, Korner R, Vincenz-Donnelly L, Hayer-Hartl M, Hartl FU. 2016. Failure of RQC machinery causes protein aggregation and proteotoxic stress. *Nature* **531**: 191-195.
- Chu J, Hong NA, Masuda CA, Jenkins BV, Nelms KA, Goodnow CC, Glynne RJ, Wu H, Masliah E, Joazeiro CA et al. 2009. A mouse forward genetics screen identifies LISTERIN as an E3 ubiquitin ligase involved in neurodegeneration. *Proc Natl Acad Sci U S A* **106**: 2097-2103.
- Cohen-Kaplan V, Livneh I, Avni N, Cohen-Rosenzweig C, Ciechanover A. 2016. The ubiquitin-proteasome system and autophagy: Coordinated and independent activities. *Int J Biochem Cell Biol* **79**: 403-418.
- Collins GA, Goldberg AL. 2017. The logic of the 26S proteasome. *Cell* **169**: 792-806.
- Das I, Krzyzosiak A, Schneider K, Wrabetz L, D'Antonio M, Barry N, Sigurdardottir A, Bertolotti A. 2015. Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science* **348**: 239-242.
- de la Pena AH, Goodall EA, Gates SN, Lander GC, Martin A. 2018. Substrate-engaged 26S proteasome structures reveal mechanisms for ATP-hydrolysis-driven translocation. *Science* **362**.
- Deuerling E, Schulze-Specking A, Tomoyasu T, Mogk A, Bukau B. 1999. Trigger factor and DnaK cooperate in folding of newly synthesized proteins. *Nature* **400**: 693-696.
- Dikic I. 2017. Proteasomal and autophagic degradation systems. *Annu Rev Biochem* **86**: 193-224.
- Dillin A, Cohen E. 2011. Ageing and protein aggregation-mediated disorders: from invertebrates to mammals. *Philos Trans R Soc Lond B Biol Sci* **366**: 94-98.
- Dobson CM, Karplus M. 1999. The fundamentals of protein folding: bringing together theory and experiment. *Curr Opin Struct Biol* **9**: 92-101.
- Doma MK, Parker R. 2007. RNA quality control in eukaryotes. *Cell* **131**: 660-668.
- Dong Y, Zhang S, Wu Z, Li X, Wang WL, Zhu Y, Stoilova-McPhie S, Lu Y, Finley D, Mao Y. 2019. Cryo-EM structures and dynamics of substrate-engaged human 26S proteasome. *Nature* **565**: 49-55.
- Dunker AK, Silman I, Uversky VN, Sussman JL. 2008. Function and structure of inherently disordered proteins. *Curr Opin Struct Biol* **18**: 756-764.
- Ellis RJ, Minton AP. 2006. Protein aggregation in crowded environments. *Biol Chem* **387**: 485-497.
- Esser C, Alberti S, Hohfeld J. 2004. Cooperation of molecular chaperones with the ubiquitin/proteasome system. *Biochim Biophys Acta* **1695**: 171-188.
- Finley D. 2009. Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu Rev Biochem* **78**: 477-513.

- Finley D, Ulrich HD, Sommer T, Kaiser P. 2012. The ubiquitin-proteasome system of *Saccharomyces cerevisiae*. *Genetics* **192**: 319-360.
- Forster F, Unverdorben P, Sledz P, Baumeister W. 2013. Unveiling the long-held secrets of the 26S proteasome. *Structure* **21**: 1551-1562.
- Frydman J. 2001. Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu Rev Biochem* **70**: 603-647.
- Frydman J, Nimmegern E, Ohtsuka K, Hartl FU. 1994. Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones. *Nature* **370**: 111-117.
- Fu H, Hardy J, Duff KE. 2018. Selective vulnerability in neurodegenerative diseases. *Nat Neurosci* **21**: 1350-1358.
- Fujimuro M, Sawada H, Yokosawa H. 1997. Dynamics of ubiquitin conjugation during heat-shock response revealed by using a monoclonal antibody specific to multi-ubiquitin chains. *Eur J Biochem* **249**: 427-433.
- Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cecconi F, Choi AM, Chu CT, Codogno P, Colombo MI et al. 2017. Molecular definitions of autophagy and related processes. *EMBO J* **36**: 1811-1836.
- Gamerding M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C. 2009. Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J* **28**: 889-901.
- Gao X, Carroni M, Nussbaum-Krammer C, Mogk A, Nillegoda NB, Szlachcic A, Guilbride DL, Saibil HR, Mayer MP, Bukau B. 2015. Human Hsp70 disaggregase reverses Parkinson's-linked alpha-Synuclein amyloid fibrils. *Mol Cell* **59**: 781-793.
- Garcia-Prat L, Martinez-Vicente M, Perdiguero E, Ortet L, Rodriguez-Ubreva J, Rebollo E, Ruiz-Bonilla V, Gutarra S, Ballestar E, Serrano AL et al. 2016. Autophagy maintains stemness by preventing senescence. *Nature* **529**: 37-42.
- Garcia-Prat L, Sousa-Victor P, Munoz-Canoves P. 2017. Proteostatic and metabolic control of stemness. *Cell Stem Cell* **20**: 593-608.
- Geiler-Samerotte KA, Dion MF, Budnik BA, Wang SM, Hartl DL, Drummond DA. 2011. Misfolded proteins impose a dosage-dependent fitness cost and trigger a cytosolic unfolded protein response in yeast. *Proc Natl Acad Sci U S A* **108**: 680-685.
- Gidalevitz T, Prahlad V, Morimoto RI. 2011. The stress of protein misfolding: from single cells to multicellular organisms. *Cold Spring Harb Perspect Biol* **3**.
- Gong Y, Kakiyama Y, Krogan N, Greenblatt J, Emili A, Zhang Z, Houry WA. 2009. An atlas of chaperone-protein interactions in *Saccharomyces cerevisiae*: implications to protein folding pathways in the cell. *Mol Syst Biol* **5**: 275.
- Guo Q, Lehmer C, Martinez-Sanchez A, Rudack T, Beck F, Hartmann H, Perez-Berlanga M, Frottin F, Hipp MS, Hartl FU et al. 2018. In situ structure of neuronal C9orf72 poly-GA aggregates reveals proteasome recruitment. *Cell* **172**: 696-705 e612.
- Hartl FU. 1996. Molecular chaperones in cellular protein folding. *Nature* **381**: 571-579.
- Hartl FU, Bracher A, Hayer-Hartl M. 2011. Molecular chaperones in protein folding and proteostasis. *Nature* **475**: 324-332.
- Hartl FU, Hayer-Hartl M. 2009. Converging concepts of protein folding in vitro and in vivo. *Nat Struct Mol Biol* **16**: 574-581.
- Hayer-Hartl M, Bracher A, Hartl FU. 2016. The GroEL-GroES chaperonin machine: A nanocage for protein folding. *Trends Biochem Sci* **41**: 62-76.
- Hetz C, Chevet E, Oakes SA. 2015. Proteostasis control by the unfolded protein response. *Nat Cell Biol* **17**: 829-838.

- Higuchi-Sanabria R, Frankino PA, Paul JW, 3rd, Tronnes SU, Dillin A. 2018. A futile battle? Protein quality control and the stress of aging. *Dev Cell* **44**: 139-163.
- Hipp MS, Park SH, Hartl FU. 2014. Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends Cell Biol* **24**: 506-514.
- Hipp MS, Patel CN, Bersuker K, Riley BE, Kaiser SE, Shaler TA, Brandeis M, Kopito RR. 2012. Indirect inhibition of 26S proteasome activity in a cellular model of Huntington's disease. *J Cell Biol* **196**: 573-587.
- Holtkamp W, Kokic G, Jager M, Mittelstaet J, Komar AA, Rodnina MV. 2015. Cotranslational protein folding on the ribosome monitored in real time. *Science* **350**: 1104-1107.
- Iadanza MG, Jackson MP, Hewitt EW, Ranson NA, Radford SE. 2018. A new era for understanding amyloid structures and disease. *Nat Rev Mol Cell Biol*.
- Ingolia NT, Lareau LF, Weissman JS. 2011. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell* **147**: 789-802.
- Isken O, Maquat LE. 2007. Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. *Genes Dev* **21**: 1833-1856.
- Izawa T, Park SH, Zhao L, Hartl FU, Neupert W. 2017. Cytosolic protein Vms1 links ribosome quality control to mitochondrial and cellular homeostasis. *Cell* **171**: 890-903 e818.
- Joazeiro CAP. 2017. Ribosomal stalling during translation: Providing substrates for ribosome-associated protein quality control. *Annu Rev Cell Dev Biol* **33**: 343-368.
- Kakkar V, Mansson C, de Mattos EP, Bergink S, van der Zwaag M, van Waarde M, Kloosterhuis NJ, Melki R, van Cruchten RTP, Al-Karadaghi S et al. 2016. The S/T-rich motif in the DNAJB6 chaperone delays polyglutamine aggregation and the onset of disease in a mouse model. *Mol Cell* **62**: 272-283.
- Kampinga HH, Craig EA. 2010. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* **11**: 579-592.
- Kaushik S, Cuervo AM. 2018. The coming of age of chaperone-mediated autophagy. *Nat Rev Mol Cell Biol* **19**: 365-381.
- Kerner MJ, Naylor DJ, Ishihama Y, Maier T, Chang HC, Stines AP, Georgopoulos C, Frishman D, Hayer-Hartl M, Mann M et al. 2005. Proteome-wide analysis of chaperonin-dependent protein folding in Escherichia coli. *Cell* **122**: 209-220.
- Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU. 2013. Molecular chaperone functions in protein folding and proteostasis. *Annu Rev Biochem* **82**: 323-355.
- Klaips CL, Jayaraj GG, Hartl FU. 2018. Pathways of cellular proteostasis in aging and disease. *J Cell Biol* **217**: 51-63.
- Knoblich JA. 2008. Mechanisms of asymmetric stem cell division. *Cell* **132**: 583-597.
- Krakowiak J, Zheng X, Patel N, Feder ZA, Anandhakumar J, Valerius K, Gross DS, Khalil AS, Pincus D. 2018. Hsf1 and Hsp70 constitute a two-component feedback loop that regulates the yeast heat shock response. *Elife* **7**.
- Kramer G, Shiber A, Bukau B. 2018. Mechanisms of cotranslational maturation of newly synthesized proteins. *Annu Rev Biochem*.
- Kulak NA, Geyer PE, Mann M. 2017. Loss-less nano-fractionator for high sensitivity, high coverage proteomics. *Mol Cell Proteomics* **16**: 694-705.
- Kumar L, Futschik ME. 2007. Mfuzz: a software package for soft clustering of microarray data. *Bioinformatics* **2**: 5-7.
- Kumsta C, Chang JT, Schmalz J, Hansen M. 2017. Hormetic heat stress and HSF-1 induce autophagy to improve survival and proteostasis in *C. elegans*. *Nat Commun* **8**: 14337.

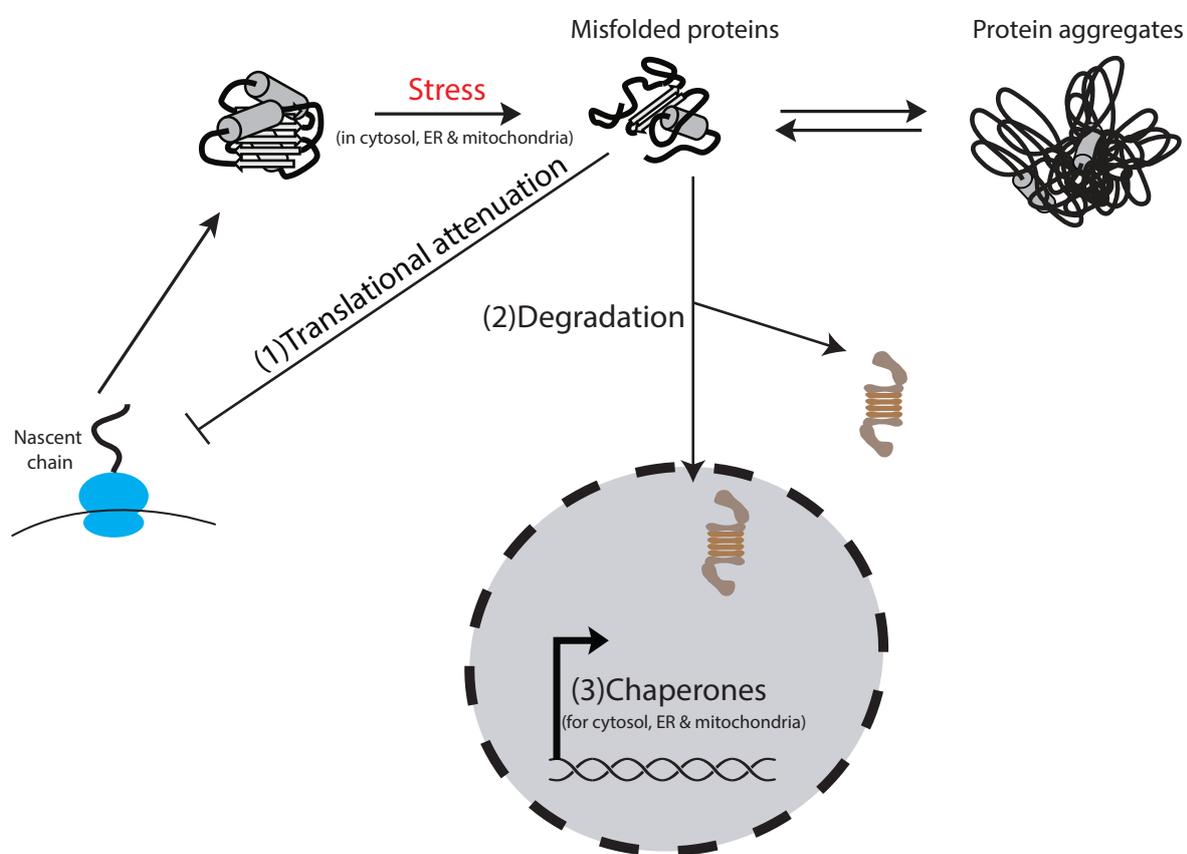
- Kundrat L, Regan L. 2010. Balance between folding and degradation for Hsp90-dependent client proteins: a key role for CHIP. *Biochemistry* **49**: 7428-7438.
- Kuo SY, Castoreno AB, Aldrich LN, Lassen KG, Goel G, Dancik V, Kuballa P, Latorre I, Conway KL, Sarkar S et al. 2015. Small-molecule enhancers of autophagy modulate cellular disease phenotypes suggested by human genetics. *Proc Natl Acad Sci U S A* **112**: E4281-4287.
- Labbadia J, Cunliffe H, Weiss A, Katsyuba E, Sathasivam K, Seredenina T, Woodman B, Moussaoui S, Frentzel S, Luthi-Carter R et al. 2011. Altered chromatin architecture underlies progressive impairment of the heat shock response in mouse models of Huntington disease. *J Clin Invest* **121**: 3306-3319.
- Labbadia J, Morimoto RI. 2015. The biology of proteostasis in aging and disease. *Annu Rev Biochem* **84**: 435-464.
- Labbadia J, Novoselov SS, Bett JS, Weiss A, Paganetti P, Bates GP, Cheetham ME. 2012. Suppression of protein aggregation by chaperone modification of high molecular weight complexes. *Brain* **135**: 1180-1196.
- Langer T, Lu C, Echols H, Flanagan J, Hayer MK, Hartl FU. 1992. Successive action of DnaK, DnaJ and GroEL along the pathway of chaperone-mediated protein folding. *Nature* **356**: 683-689.
- Lee BH, Lee MJ, Park S, Oh DC, Elsasser S, Chen PC, Gartner C, Dimova N, Hanna J, Gygi SP et al. 2010. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* **467**: 179-184.
- Leestemaker Y, de Jong A, Witting KF, Penning R, Schuurman K, Rodenko B, Zaal EA, van de Kooij B, Laufer S, Heck AJR et al. 2017. Proteasome activation by small molecules. *Cell Chem Biol* **24**: 725-736 e727.
- Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A, Chanda SK, Batalov S, Joazeiro CA. 2008. Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. *PLoS One* **3**: e1487.
- Liu B, Han Y, Qian SB. 2013. Cotranslational response to proteotoxic stress by elongation pausing of ribosomes. *Mol Cell* **49**: 453-463.
- Livneh I, Cohen-Kaplan V, Cohen-Rosenzweig C, Avni N, Ciechanover A. 2016. The life cycle of the 26S proteasome: from birth, through regulation and function, and onto its death. *Cell Res* **26**: 869-885.
- Llorens-Bobadilla E, Zhao S, Baser A, Saiz-Castro G, Zwadlo K, Martin-Villalba A. 2015. Single-cell transcriptomics reveals a population of dormant neural stem cells that become activated upon brain injury. *Cell Stem Cell* **17**: 329-340.
- Mahat DB, Salamanca HH, Duarte FM, Danko CG, Lis JT. 2016. Mammalian heat shock response and mechanisms underlying its genome-wide transcriptional regulation. *Mol Cell* **62**: 63-78.
- Medicherla B, Goldberg AL. 2008. Heat shock and oxygen radicals stimulate ubiquitin-dependent degradation mainly of newly synthesized proteins. *J Cell Biol* **182**: 663-673.
- Melber A, Haynes CM. 2018. UPR(mt) regulation and output: a stress response mediated by mitochondrial-nuclear communication. *Cell Res* **28**: 281-295.
- Mevissen TET, Komander D. 2017. Mechanisms of deubiquitinase specificity and regulation. *Annu Rev Biochem* **86**: 159-192.
- Milo R. 2013. What is the total number of protein molecules per cell volume? A call to rethink some published values. *Bioessays* **35**: 1050-1055.

- Moehle EA, Shen K, Dillin A. 2018. Mitochondrial proteostasis in the context of cellular and organismal health and aging. *J Biol Chem*.
- Mogk A, Bukau B, Kampinga HH. 2018. Cellular handling of protein aggregates by disaggregation machines. *Mol Cell* **69**: 214-226.
- Mogk A, Kummer E, Bukau B. 2015. Cooperation of Hsp70 and Hsp100 chaperone machines in protein disaggregation. *Front Mol Biosci* **2**: 22.
- Morimoto RI. 2011. The heat shock response: systems biology of proteotoxic stress in aging and disease. *Cold Spring Harb Symp Quant Biol* **76**: 91-99.
- Muchowski PJ, Schaffar G, Sittler A, Wanker EE, Hayer-Hartl MK, Hartl FU. 2000. Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc Natl Acad Sci U S A* **97**: 7841-7846.
- Mymrikov EV, Daake M, Richter B, Haslbeck M, Buchner J. 2017. The chaperone activity and substrate spectrum of human small heat shock proteins. *J Biol Chem* **292**: 672-684.
- Nagy M, Fenton WA, Li D, Furtak K, Horwich AL. 2016. Extended survival of misfolded G85R SOD1-linked ALS mice by transgenic expression of chaperone Hsp110. *Proc Natl Acad Sci U S A* **113**: 5424-5428.
- Nedialkova DD, Leidel SA. 2015. Optimization of codon translation rates via tRNA modifications maintains proteome integrity. *Cell* **161**: 1606-1618.
- Neef DW, Jaeger AM, Gomez-Pastor R, Willmund F, Frydman J, Thiele DJ. 2014. A direct regulatory interaction between chaperonin TRiC and stress-responsive transcription factor HSF1. *Cell Rep* **9**: 955-966.
- Nguyen AT, Prado MA, Schmidt PJ, Sendamarai AK, Wilson-Grady JT, Min M, Campagna DR, Tian G, Shi Y, Dederer V et al. 2017. UBE2O remodels the proteome during terminal erythroid differentiation. *Science* **357**.
- Nillegoda NB, Kirstein J, Szlachcic A, Berynsky M, Stank A, Stengel F, Arnsburg K, Gao X, Scior A, Aebersold R et al. 2015. Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. *Nature* **524**: 247-251.
- Nilsson OB, Hedman R, Marino J, Wickles S, Bischoff L, Johansson M, Muller-Lucks A, Trovato F, Puglisi JD, O'Brien EP et al. 2015. Cotranslational protein folding inside the ribosome exit tunnel. *Cell Rep* **12**: 1533-1540.
- Noormohammadi A, Khodakarami A, Gutierrez-Garcia R, Lee HJ, Koyuncu S, Konig T, Schindler C, Saez I, Fatima A, Dieterich C et al. 2016. Somatic increase of CCT8 mimics proteostasis of human pluripotent stem cells and extends *C. elegans* lifespan. *Nat Commun* **7**: 13649.
- Oh E, Becker AH, Sandikci A, Huber D, Chaba R, Gloge F, Nichols RJ, Typas A, Gross CA, Kramer G et al. 2011. Selective ribosome profiling reveals the cotranslational chaperone action of trigger factor in vivo. *Cell* **147**: 1295-1308.
- Olzscha H, Schermann SM, Woerner AC, Pinkert S, Hecht MH, Tartaglia GG, Vendruscolo M, Hayer-Hartl M, Hartl FU, Vabulas RM. 2011. Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* **144**: 67-78.
- Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM. 2016. The integrated stress response. *EMBO Rep* **17**: 1374-1395.
- Parag HA, Raboy B, Kulka RG. 1987. Effect of heat shock on protein degradation in mammalian cells: involvement of the ubiquitin system. *EMBO J* **6**: 55-61.
- Park SH, Kukushkin Y, Gupta R, Chen T, Konagai A, Hipp MS, Hayer-Hartl M, Hartl FU. 2013. PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. *Cell* **154**: 134-145.
- Parsell DA, Kowal AS, Singer MA, Lindquist S. 1994. Protein disaggregation mediated by heat-shock protein Hsp104. *Nature* **372**: 475-478.

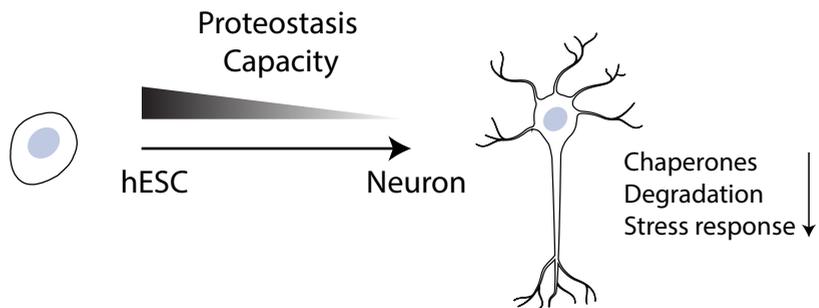
- Powers ET, Balch WE. 2013. Diversity in the origins of proteostasis networks--a driver for protein function in evolution. *Nat Rev Mol Cell Biol* **14**: 237-248.
- Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. 2009. Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem* **78**: 959-991.
- Preissler S, Deuerling E. 2012. Ribosome-associated chaperones as key players in proteostasis. *Trends Biochem Sci* **37**: 274-283.
- Raychaudhuri S, Loew C, Korner R, Pinkert S, Theis M, Hayer-Hartl M, Buchholz F, Hartl FU. 2014. Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1. *Cell* **156**: 975-985.
- Richter K, Haslbeck M, Buchner J. 2010. The heat shock response: Life on the verge of death. *Molecular Cell* **40**: 253-266.
- Rizzolo K, Huen J, Kumar A, Phanse S, Vlasblom J, Kakiyama Y, Zeineddine HA, Minic Z, Snider J, Wang W et al. 2017. Features of the chaperone cellular network revealed through systematic Interaction mapping. *Cell Rep* **20**: 2735-2748.
- Rodina A, Wang T, Yan P, Gomes ED, Dunphy MP, Pillarsetty N, Koren J, Gerecitano JF, Taldone T, Zong H et al. 2016. The epichaperome is an integrated chaperome network that facilitates tumour survival. *Nature* **538**: 397-401.
- Roth DM, Hutt DM, Tong J, Bouhcecareilh M, Wang N, Seeley T, Dekkers JF, Beekman JM, Garza D, Drew L et al. 2014. Modulation of the maladaptive stress response to manage diseases of protein folding. *PLoS Biol* **12**: e1001998.
- Rubinsztein DC. 2006. The roles of intracellular protein-degradation pathways in neurodegeneration. *Nature* **443**: 780-786.
- Sala AJ, Bott LC, Morimoto RI. 2017. Shaping proteostasis at the cellular, tissue, and organismal level. *J Cell Biol* **216**: 1231-1241.
- Saretzki G, Armstrong L, Leake A, Lako M, von Zglinicki T. 2004. Stress defense in murine embryonic stem cells is superior to that of various differentiated murine cells. *Stem Cells* **22**: 962-971.
- Saretzki G, Walter T, Atkinson S, Passos JF, Bareth B, Keith WN, Stewart R, Hoare S, Stojkovic M, Armstrong L et al. 2008. Downregulation of multiple stress defense mechanisms during differentiation of human embryonic stem cells. *Stem Cells* **26**: 455-464.
- Sarkar S, Ravikumar B, Floto RA, Rubinsztein DC. 2009. Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. *Cell Death Differ* **16**: 46-56.
- Scior A, Buntru A, Arnsburg K, Ast A, Iburg M, Juenemann K, Pigazzini ML, Mlody B, Puchkov D, Priller J et al. 2018. Complete suppression of Htt fibrilization and disaggregation of Htt fibrils by a trimeric chaperone complex. *EMBO J* **37**: 282-299.
- Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen T, Heckl D, Ebert BL, Root DE, Doench JG et al. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**: 84-87.
- Shalgi R, Hurt JA, Krykbaeva I, Taipale M, Lindquist S, Burge CB. 2013. Widespread regulation of translation by elongation pausing in heat shock. *Mol Cell* **49**: 439-452.
- Signer RA, Magee JA, Salic A, Morrison SJ. 2014. Haematopoietic stem cells require a highly regulated protein synthesis rate. *Nature* **509**: 49-54.
- Sittler A, Lurz R, Lueder G, Priller J, Lehrach H, Hayer-Hartl MK, Hartl FU, Wanker EE. 2001. Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum Mol Genet* **10**: 1307-1315.
- Solis EJ, Pandey JP, Zheng X, Jin DX, Gupta PB, Airoidi EM, Pincus D, Denic V. 2016. Defining the essential function of yeast Hsf1 reveals a compact transcriptional program for maintaining eukaryotic proteostasis. *Mol Cell* **63**: 60-71.

- Spriggs KA, Bushell M, Willis AE. 2010. Translational regulation of gene expression during conditions of cell stress. *Mol Cell* **40**: 228-237.
- Suraweera A, Munch C, Hanssum A, Bertolotti A. 2012. Failure of amino acid homeostasis causes cell death following proteasome inhibition. *Mol Cell* **48**: 242-253.
- Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, Karras GI, Lindquist S. 2012. Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. *Cell* **150**: 987-1001.
- Taipale M, Tucker G, Peng J, Krykbaeva I, Lin ZY, Larsen B, Choi H, Berger B, Gingras AC, Lindquist S. 2014. A quantitative chaperone interaction network reveals the architecture of cellular protein homeostasis pathways. *Cell* **158**: 434-448.
- Taylor RC, Dillin A. 2011. Aging as an event of proteostasis collapse. *Cold Spring Harb Perspect Biol* **3**.
- Teter SA, Houry WA, Ang D, Tradler T, Rockabrand D, Fischer G, Blum P, Georgopoulos C, Hartl FU. 1999. Polypeptide flux through bacterial Hsp70: DnaK cooperates with trigger factor in chaperoning nascent chains. *Cell* **97**: 755-765.
- Thibautaud TA, Anderson RT, Smith DM. 2018. A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers. *Nat Commun* **9**: 1097.
- Tsaytler P, Harding HP, Ron D, Bertolotti A. 2011. Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science* **332**: 91-94.
- Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A et al. 2015. Proteomics. Tissue-based map of the human proteome. *Science* **347**: 1260419.
- Vabulas RM, Raychaudhuri S, Hayer-Hartl M, Hartl FU. 2010. Protein folding in the cytoplasm and the heat shock response. *Cold Spring Harb Perspect Biol* **2**: a004390.
- Varshavsky A. 2017. The ubiquitin system, autophagy, and regulated protein degradation. *Annu Rev Biochem* **86**: 123-128.
- Vilchez D, Boyer L, Morantte I, Lutz M, Merkwirth C, Joyce D, Spencer B, Page L, Masliah E, Berggren WT et al. 2012. Increased proteasome activity in human embryonic stem cells is regulated by PSMD11. *Nature* **489**: 304-308.
- Vilchez D, Simic MS, Dillin A. 2014. Proteostasis and aging of stem cells. *Trends Cell Biol* **24**: 161-170.
- Wallace EW, Kear-Scott JL, Pilipenko EV, Schwartz MH, Laskowski PR, Rojek AE, Katanski CD, Riback JA, Dion MF, Franks AM et al. 2015. Reversible, specific, active aggregates of endogenous proteins assemble upon heat stress. *Cell* **162**: 1286-1298.
- Walter P, Ron D. 2011. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334**: 1081-1086.
- Watanabe Y, Tsujimura A, Taguchi K, Tanaka M. 2017. HSF1 stress response pathway regulates autophagy receptor SQSTM1/p62-associated proteostasis. *Autophagy* **13**: 133-148.
- Westerheide SD, Anckar J, Stevens SM, Jr., Sistonen L, Morimoto RI. 2009. Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science* **323**: 1063-1066.
- Wilhelm M, Schlegl J, Hahne H, Gholami AM, Lieberenz M, Savitski MM, Ziegler E, Butzmann L, Gessulat S, Marx H et al. 2014. Mass-spectrometry-based draft of the human proteome. *Nature* **509**: 582-587.
- Willmund F, del Alamo M, Pechmann S, Chen T, Albanese V, Dammer EB, Peng J, Frydman J. 2013. The cotranslational function of ribosome-associated Hsp70 in eukaryotic protein homeostasis. *Cell* **152**: 196-209.

- Wolff S, Weissman JS, Dillin A. 2014. Differential scales of protein quality control. *Cell* **157**: 52-64.
- Xu G, Pattamatta A, Hildago R, Pace MC, Brown H, Borchelt DR. 2016. Vulnerability of newly synthesized proteins to proteostasis stress. *J Cell Sci* **129**: 1892-1901.
- Yam AY, Xia Y, Lin HT, Burlingame A, Gerstein M, Frydman J. 2008. Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. *Nat Struct Mol Biol* **15**: 1255-1262.
- Yanagitani K, Juszkievicz S, Hegde RS. 2017. UBE2O is a quality control factor for orphans of multiprotein complexes. *Science* **357**: 472-475.
- Yang J, Oza J, Bridges K, Chen KY, Liu AY. 2008. Neural differentiation and the attenuated heat shock response. *Brain Res* **1203**: 39-50.
- Yao Z, Mich JK, Ku S, Menon V, Krostag AR, Martinez RA, Furchtgott L, Mulholland H, Bort S, Fuqua MA et al. 2017. A single-cell roadmap of lineage bifurcation in human ESC models of embryonic brain development. *Cell Stem Cell* **20**: 120-134.
- Yonashiro R, Tahara EB, Bengtson MH, Khokhrina M, Lorenz H, Chen KC, Kigoshi-Tansho Y, Savas JN, Yates JR, Kay SA et al. 2016. The Rqc2/Tae2 subunit of the ribosome-associated quality control (RQC) complex marks ribosome-stalled nascent polypeptide chains for aggregation. *Elife* **5**: e11794.
- You KT, Park J, Kim VN. 2015. Role of the small subunit processome in the maintenance of pluripotent stem cells. *Genes Dev* **29**: 2004-2009.
- Yu A, Shibata Y, Shah B, Calamini B, Lo DC, Morimoto RI. 2014. Protein aggregation can inhibit clathrin-mediated endocytosis by chaperone competition. *Proc Natl Acad Sci USA* **111**: E1481-1490.
- Zaher HS, Green R. 2009. Fidelity at the molecular level: lessons from protein synthesis. *Cell* **136**: 746-762.
- Zhang T, Shen S, Qu J, Ghaemmaghani S. 2016. Global analysis of cellular protein flux quantifies the selectivity of basal autophagy. *Cell Rep* **14**: 2426-2439.
- Zhao J, Zhai B, Gygi SP, Goldberg AL. 2015. mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy. *Proc Natl Acad Sci USA* **112**: 15790-15797.
- Zhao R, Davey M, Hsu YC, Kaplanek P, Tong A, Parsons AB, Krogan N, Cagney G, Mai D, Greenblatt J et al. 2005. Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. *Cell* **120**: 715-727.
- Zheng N, Shabek N. 2017. Ubiquitin ligases: Structure, function, and regulation. *Annu Rev Biochem* **86**: 129-157.
- Zheng X, Krakowiak J, Patel N, Beyzavi A, Ezike J, Khalil AS, Pincus D. 2016. Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation. *Elife* **5**.
- Zismanov V, Chichkov V, Colangelo V, Jamet S, Wang S, Syme A, Koromilas AE, Crist C. 2016. Phosphorylation of eIF2alpha is a translational control mechanism regulating muscle stem cell quiescence and self-renewal. *Cell Stem Cell* **18**: 79-90.
- Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R. 1998. Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell* **94**: 471-480.
- Zwirowski S, Klosowska A, Obuchowski I, Nillegoda NB, Pirog A, Zietkiewicz S, Bukau B, Mogk A, Liberek K. 2017. Hsp70 displaces small heat shock proteins from aggregates to initiate protein refolding. *EMBO J* **36**: 783-796.



A



B

