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Perspective

Proteostasis and the Aging Proteome in Health and Disease

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The maintenance of the proteome is essential to preserve cell functionality and the ability to respond and adapt to the changing environment. This is regulated by the proteostasis network, a dedicated set of molecular components comprised of molecular chaperones and protein clearance mechanisms, regulated by cell stress signaling pathways, that prevents the toxicity associated with protein misfolding and accumulation of toxic aggregates in different subcellular compartments and tissues. The efficiency of the proteostasis network declines with age and this failure in protein homeostasis has been proposed to underlie the basis of common age-related human disorders. The current advances in the understanding of the mechanisms and regulation of proteostasis and of the different types of digressions in this process in aging have turned the attention toward the therapeutic opportunities offered by the restoration of proteostasis in age-associated degenerative diseases. Here, we discuss some of the unresolved questions on proteostasis that need to be addressed to enhance healthspan and to diminish the pathology associated with persistent protein damage.

Key Words: Autophagy—Chaperones—ER stress—Human degenerative diseases—Proteasome.

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THE presence of intra- and extracellular deposits of proteins affected by altered conformation and post-translational modifications, a feature shared by many common human disorders, has contributed to the current awareness of the importance of protein homeostasis to proper cellular functioning and for cells to adapt to the demands imposed by environmental and physiological stress (1,2). Molecular damage during protein synthesis, folding and assembly, and trafficking and clearance are associated in a broad range of diseases known as protein conformational diseases that include neurodegeneration, cancer, and immunological and metabolic diseases (3). It is not coincidental that a common characteristic of most of these protein conformational diseases is their higher incidence later in life and that aging is considered as a major risk factor. The gradual inability of cells and organisms to maintain protein homeostasis (proteostasis) as they age has been proposed to contribute to their overall loss of fitness, inadequate response to stress, and reduced healthspan (2,4,5). This link between faulty proteostasis in aging and higher incidence of disease explains the current interest to explore the therapeutic opportunities that this area has to offer to extend human healthspan. Can defects in the proteostasis network (PN) with age explain the higher disease incidence? How many diseases have underlying defects in protein homeostasis? Because the different molecular pathways involved in proteostasis are dynamic and intricately linked, we may need to take both a detailed pathway approach to identify specific faulty components in addition to a systems approach. To be able to answer these and other questions regarding the contribution of alterations in proteostasis to aging and disease, we need to address the fundamental gaps in our understanding of the mechanisms that regulate proteostasis.

All cells express an exquisitely regulated network of molecular components and cellular pathways that work coordinately to assure proteostasis (6–10) (Figure 1A). The need for this dedicated system stems from the sensitivity of proteins to intra- and extracellular stressors that challenge the stability of protein conformation, leading to protein damage, unfolding, alternate conformations, and aggregation. In addition to these stress-related situations, all intracellular proteins undergo conformational changes under physiological conditions tightly related to their functionality. Moreover, proteins are highly dynamic and are constantly undergoing folding, assembly and disassembly, and trafficking through subcellular compartments, both within and outside the cell. Each of these steps requires limited or complete structural rearrangements in protein conformation often leading to the transient exposure of

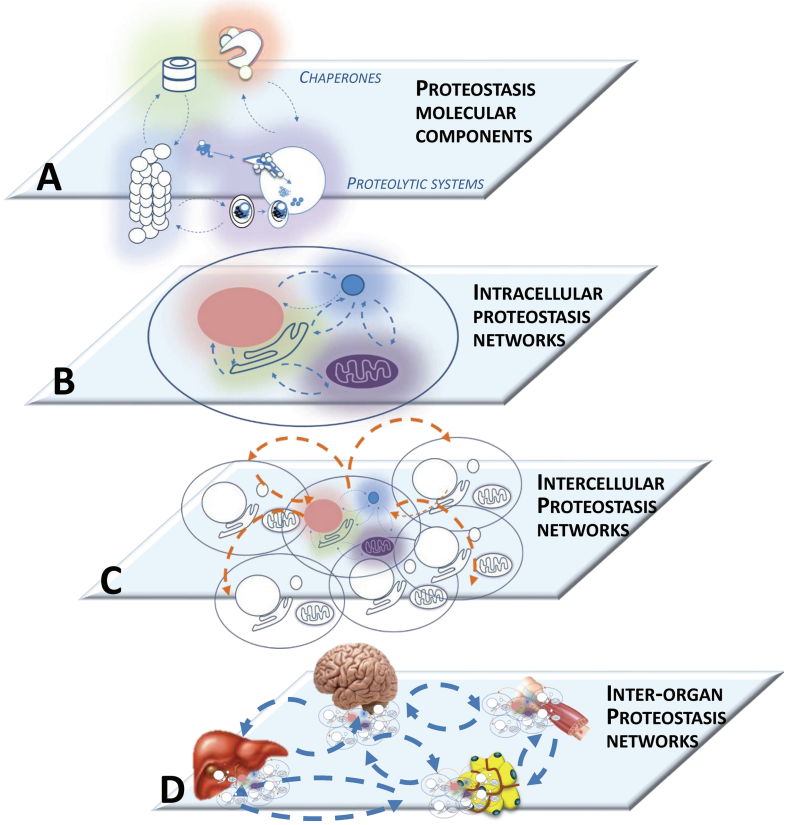

Figure 1. Schematic of the different levels of integration of the proteostasis networks. (**A**) Chaperones and proteolytic systems like the Ubiquitin Proteasome System or autophagy are the main molecular components of the systems for protein quality control. (**B**) Dedicated mechanisms are in place in almost all organelles to assure homeostasis of their subproteome. These organelle-specific systems feed back into each other for coordinate maintenance of cellular homeostasis. (**C** and **D**) Although still poorly understood, the proteostasis networks of one cell have an impact on those from neighboring cells (**C**) and both paracrine and endocrine molecules assure the coordinated functioning and reactivity of the proteostasis network across organs and systems (**D**) to attain an integrated response to stress and conditions that challenge protein homeostasis.

hydrophobic protein domains, usually buried within the core of the protein and a multitude of intermolecular interactions with intrinsically disordered and metastable domains (3). The risk of exposure of these aggregation-prone regions in the proaggregating cellular milieu makes absolutely essential the intervention of chaperones and proteolytic systems of the PN. These cellular networks are highly regulated and respond to a complexity of inputs from the extracellular and intracellular milieu. Moreover, regulation of the PN occurs at multiple levels that extend beyond the individual cell to interactions between tissues via cell nonautonomous control that functions at the level of the organism. The dedicated subsets of chaperones and proteases responsible for the maintenance of proteostasis in specific subcellular compartments and organelles are in constant intercommunication (Figure 1B). For example, the rapid adjustment of the cytosolic PNs in response to proteotoxicity in a specific organelle permits cooperation between these networks to help resolve the problem and rapidly return to homeostasis. However, this cooperation does not end at the cellular boundaries. Altered proteostasis in a cell elicits a response in neighboring cells that prepares them to cope better with proteotoxicity-inducing insults (11) (Figure 1C). Added to this paracrine regulation, it has been recently revealed that changes in proteostasis of one organ have profound effects on the PNs of distant organs (12,13) (Figure 1D). This systemic regulation of organismal proteostasis could offer unique opportunities to restore homeostasis and consequently function in an organ by intervening in another organ more accessible or easier to manipulate (14–16).

The advances in the molecular dissection of the complexity of the PN, the growing evidence linking failure of these networks to human diseases, and the growing understanding of the contribution of the PN to healthspan and longevity justify the efforts to integrate these areas to accelerate discovery and to take maximal advantage of the translational opportunities from modulation of proteostasis.

Does Aging Impair the PN Leading to Proteotoxic Stress?

The Ubiquitin Proteasome System contributes to protein turnover and is an essential component of the primary defense against the accumulation of misfolded, potentially

toxic proteins (17). Comprised of more than 700 enzymes involved in ubiquitin tagging of proteins for destruction by the proteasome, they act coordinately with the major chaperones in the selective elimination of many disease-related abnormal proteins.

Major advances have been made recently in our understanding of 26S proteasome function, and it is now clear that this system can be enhanced or inhibited by pharmacologically active small molecules (9). Ubiquitination also targets endocytosed proteins and organelles (eg, mitochondria and aggregates) for lysosomal degradation, and the capacity for macroautophagy and degradation by the Ubiquitin Proteasome System are coordinately regulated (18) (Figure 1A).

Although the effects of aging on the functional capacity of the Ubiquitin Proteasome System in different tissues are not well understood, nevertheless there is increasing evidence that the 26S proteasome is compromised in certain proteotoxic disease models thus offering intriguing opportunities for therapeutic strategies (9). Likewise, there is evidence to support alterations in autophagy in major neurodegenerative and metabolic disorders associated with aging and in the loss of fitness of the musculoskeletal system with age (19–21). Moreover, decline in both clearance systems during aging is certain to have profound consequences if cells and tissues cannot effectively clear damaged proteins and cell debris. Genetic manipulations in specific components of each of these systems have proven successful in expanding life span in invertebrates and in improving the response to stress and resistance to specific disease processes in mammals.

Many fundamental questions remain, in particular the effects of aging and the mechanisms regulating these quality control processes in different tissues need to be resolved to understand and develop rational treatments for age-related diseases.

What Are the Relationships Between Cytosolic and Organelle Proteostasis That Characterize Disease?

The emphasis in protein quality control has, for some time, emphasized the cytosol and the endoplasmic reticulum, as these subcellular compartments directly involve the bulk of protein synthesis and folding. However, quality control is not limited to de novo generated proteins, but is also important for maintenance of organellar subproteomes. It is therefore anticipated that these organelle-specific proteostasis mechanisms do not function as individual entities but rather are integrated within the PN (Figure 1B).

For example, the central role of mitochondria in energy and reactive oxygen species production subjects the mitochondria proteome to constant attack that also impairs membrane permeability (22). Quality control of mitochondria is regulated by autophagy and by the mitochondrial unfolding protein response (mtUPR). Mitochondria evolved from intracellular organisms within the host cell, leading to the proposition that disintegration of mitochondria within the cell would elicit an inflammatory response, leading to autophagy as an efficient mechanism for clearance of damaged mitochondria (23).

Factors that impair global autophagy or the selective clearance of mitochondria (mitophagy), such as aging, excess nutrition, high cholesterol, metabolic syndrome, and diabetes, have a negative impact in mitochondrial homeostasis (24). Because mitophagy and mitochondrial biogenesis are functionally linked, the failure to clear dysfunctional mitochondria via autophagy will result in their accumulation and prevent their replacement with functional components (25). Impaired mitochondrial turnover is a feature of numerous diseases including Parkinson's disease, Crohn's inflammatory bowel disease, and the damage associated with myocardial ischemia and reperfusion injury (26).

Another aspect of mitochondrial quality control is the mtUPR (27,28), which is elicited by an imbalance between nuclear-encoded and mitochondrial-encoded proteins that must be coassembled into the oxidative phosphorylation complexes. Recent work has linked longevity to the mtUPR; the same agents (fasting, rapamycin, chloramphenicol, sirtuins) that activate mtUPR also induce mitophagy; however, it is not clear whether it is the mtUPR or mitophagy that contributes to life-span extension.

The existence of dedicated mechanisms for quality control of the subproteome in other cellular compartments such as Golgi, peroxisomes, nucleus, or lysosomes themselves remains still poorly elucidated, which has limited the possibility of implicating alterations in proteostasis of these organelles with aging and age-related disorders.

How Are Proteostatic Mechanisms Regulated and How Are These Mechanisms Affected by Aging?

When originally discovered, chaperones were thought to assist in protein folding following their thermodynamic potential to the native state, and then disappear from a protein's life until such time as the protein became destabilized enroute to degradation (10). It is now increasingly clear that chaperones have far more complex and diverse functions with the proteome, as they regulate cycles of alternate conformational states that can affect protein–protein interactions, signal transduction, and transcriptional programs (6,29) (Figure 1B). Chaperones also buffer the many mutations present in the proteomes of individuals and cells in both normal and diseased states, conferring robustness to mutation and genetic variation by maintaining protein function in the face of mutations.

The emerging insight that proteostasis is a highly complex, multifaceted, and nuanced actor in cellular regulation calls for a better understanding of the chaperone and

proteostasis machineries that interact with and assemble diverse clients and substrates, to understand its roles under normal conditions and in response to stress, and how it becomes dysregulated during aging and impaired in disease. This can only occur through the integration of mechanistic, cell biological, and genetic data with physiological studies in development, aging, and disease.

The therapeutic implications of such an understanding can have an enormous potential to maintain healthspan and to delay or diminish disease. For instance, it is clear that an impairment of proteostasis during aging underlies the onset of a large set of neurodegenerative misfolding diseases. Thus, an enhancement of these pathways could have a tremendous impact in our treatment of late-onset devastating diseases. Likewise, cancer cells and viruses induce the expression of chaperones to cope with their high protein production levels and exploit their buffering capacity to allow the elevated mutation rates that enable them to escape most drugs. In these examples, decreasing chaperone capacity should deprive these disease states from achieving their major protective mechanisms. Importantly, although highly interconnected and cross-regulated, there is much division of labor among the members of the proteostatic machinery.

The tremendous therapeutic promise of proteostasis regulation calls for a better understanding of basic mechanisms and pathways and their regulation in disease, infection, and aging.

How Does Impaired Proteostasis in One Cell or Tissue Affect the Organism?

There are many challenges with intracellular protein folding that include how proteostasis is balanced and communicated between organelles, and how a stressful folding environment in one tissue affects surrounding tissues and the organism.

Protein biogenesis is specified according to the subcellular compartment where they are expressed and eventually localized. For example, protein folding in the endoplasmic reticulum (ER) is fundamentally different from folding in the cytosol. Moreover, every protein that folds in the ER has intrinsic challenges that are influenced dramatically by intracellular homeostasis and extracellular stimuli (13) (Figure 1B). Protein folding in the ER also depends on additional variables that are affected by aging and disease, such as redox status, Ca^{2+} concentration, and oligosaccharide assembly.

Very little is known how one cell type or organ responds to protein misfolding signals from another compartment (Figure 1C), but several tantalizing observations have been made (12,15). Protein folding is affected by the rate of protein synthesis, consequently anabolic signals place additional pressure on the ER to increase its folding, trafficking, and secretion potential. This is exemplified by pancreatic beta cells that respond to glucose at three times per day to increase proinsulin synthesis by 10-fold. However, excessive protein synthesis also leads to oxidative stress that can disrupt productive folding in the ER, which in turn disrupts mitochondrial function to reduce oxidative phosphorylation. These events could then lead to a metabolic crisis that has potential to be transmitted across all cell membranes (15).

Inflammation and metabolic stress, associated with aging, also affects protein folding in the ER with misfolding leading to further inflammatory stress and inflammatory cytokine stimulation in hepatocytes. This inflammatory reaction is likely to alert other cells of the presence of proteotoxic stress at a distance. The specific signature that distinguishes proteotoxic stress-driven inflammation from other inflammatory signals is currently unknown. In addition, a metabolic surplus, as occurs in a high-fat diet, challenges all cell types to increase protein synthesis rates and folding to exceed folding capacity within the ER. Accumulation of lipids in the hepatocyte is another contributor to hepatic inflammation, fibrosis, and hepatocellular carcinoma (30,31). One of the fundamental questions for future modern medicine is to understand how lifestyle affects inflammatory responses, metabolism, and the protein-folding environment in different cell types. It is likely that the interactions between these components contribute to organismal pathology (Figure 1D) and degenerative diseases associated with aging.

Can Impaired Proteostasis Be a Therapeutic Target?

The growing number of connections between dysfunction of the PN and age-related disorders has provided momentum to explore the therapeutic opportunities that manipulation of this process may offer in the retardation or alleviation of severe age-related degenerative diseases (14).

A common feature of cytosol and organelle-specific PN is the presence of different signaling arms that often act cooperatively to return the affected compartment to homeostasis. The PN can thus be selectively activated through unfolded protein response arm-specific signaling to alleviate gain-of-toxic-function diseases where excessive secretion or accumulation of misfolding and aggregation of proteins leads to amyloid diseases. Substantial progress has been made to date on the identification of novel small molecules that have the properties of arm-specific unfolding protein response activators (14).

A second example for therapeutics of protein conformational diseases focuses on a chemical strategy to achieve proteostasis, wherein small molecule kinetic stabilizers produced by structure-based drug design are employed to halt the progression of peripheral neuropathy in the human disease familial amyloid polyneuropathy linked to transthyretin amyloidosis. By having delineated the molecular mechanism of transthyretin aggregation linked to pathology, this led to the identification of a small molecule

therapeutically active compound that stabilizes transthyretin and suppresses its aggregation. These efforts have resulted in a regulatory agency approved drug and provide the first pharmacologic evidence supporting the amyloid hypothesis, the notion that protein aggregation causes degeneration of the heart and the nervous system (16). Finally, the efforts to get transthyretin-stabilizing compounds into the clinic have provided insights about the etiology of protein conformational diseases vis-à-vis a successful clinical trial and longer term patient assessments.

Closing Comments

The study of proteostasis has gained rapid momentum as a result of the growing number of connections between this process and human disease. Moreover, the alterations in proteostasis during aging offers a unifying pathogenic mechanism of the so-called age-related diseases offers now a promising rationale for the development of novel therapeutic strategies to prevent protein misfolding and aggregation and to diminish cellular and organism toxicity. Although examples of successful application of proteostasis-targeted interventions are starting to appear in the basic scientific literature, clinical implementation of these approaches as a common therapeutic for age-related disorders requires a better understanding of the intricacies and regulation of the PN. Although there has been substantial efforts to understand how these different networks function at the cellular level as separate entities in each cellular compartment, it is now the time to place the emphasis on integration and intercommunication of the PN among organelles, between cells and across systems, including extracellular paracrine- and endocrine-signaling molecules. Future studies will address the missing links necessary to apply our knowledge of proteostasis to address the many age-related diseases known or suspected to be impinged upon by changes in protein quality control with aging.

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