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Chapter 491: Protein Folding Disorders

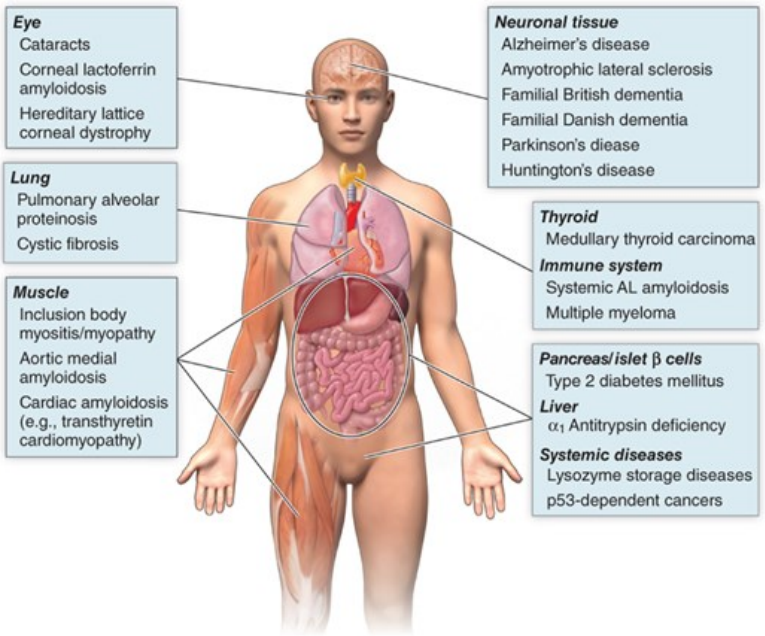
Richard I. Morimoto; G. Scott Budinger

INTRODUCTION

Many hundreds of human diseases, collectively known as protein conformational diseases or protein folding disorders, result from protein misfolding due to intrinsic and extrinsic errors amplified by exposures to environmental and physiologic stress conditions. Despite many years of considerable effort, there remains no useful algorithm that can effectively predict protein tertiary (three-dimensional) structure from primary amino acid sequence (and its variants), let alone posttranslationally modified (from environmental exposures) primary sequence. Such events challenge the integrity of the proteome and can lead to premature clearance, mislocalization, dysfunction or aggregation of proteins, thus affecting cellular robustness, health, and longevity. Mismanagement of the proteome is the basis of a broad class of hundreds of diseases that include orphan lysosomal storage diseases, type 2 diabetes, cystic fibrosis, certain fibrotic diseases, metabolic diseases, muscle wasting diseases, cancer, and neurodegenerative diseases, as exemplified by Alzheimer's disease, frontotemporal dementia, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Huntington's disease **(Fig. 491-1)**. For each of these diseases and many others described in this textbook, aging is the major contributing risk factor.

FIGURE 491-1

Diseases of protein folding. A representative list of tissues and known folding diseases.



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The challenge at the biochemical and molecular level is for the cell to achieve a stable and functional proteome during development that persists through young adulthood and to maintain it throughout aging. For humans, this is necessary for the operational health of each of the tens of trillions of cells that compose our ~80 organs for health span and life span. To achieve this goal, our cells have evolved a remarkably efficient proteostasis network (PN) composed of molecular chaperones and other highly conserved components essential for normal protein synthesis, folding, translocation, and degradation **(Fig. 491-2)** that balances input with output and that ensures every protein is functional. The PN is essential for all tissues and for the diverse protein-protein interactions required for cell signaling, biosynthetic processes, and the structural demands for tissue

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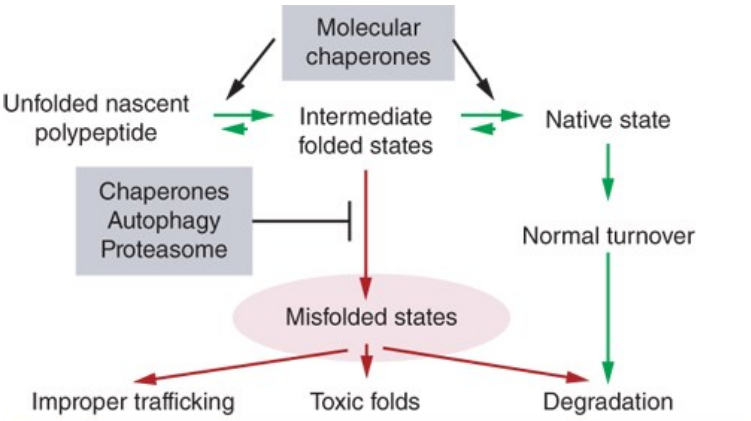
shape, mechanical properties, and function. An equal, if not more important, role for the PN is to detect, prevent, and remove misfolded and aggregated proteins that accumulate in stress, aging, and disease and that thereby interfere with cellular health. Understanding how proteostasis is achieved and maintained is of fundamental biological interest and essential to prevent age-associated protein folding disorders.

FIGURE 491-2

The proteostasis network and protein folding diseases. The process of protein biogenesis involves the action of molecular chaperones to ensure the transition of the unfolded nascent polypeptide through intermediates to the folded native state. Such proteins then have a natural turnover. Off-pathway species are prevented by the actions of chaperones and the recognition of nonnative misfolded states and aggregates by the autophagic-lysosomal machinery and the ubiquitin-proteasome. When misfolded species escape quality control, they can then become improperly trafficked, as occurs for α_1 antitrypsin associated with emphysema; for toxic folds as occurs for amyloid beta, tau, huntington, and SOD1 in amyloidogenic neurodegenerative diseases; or prematurely degraded as occurs for cystic fibrosis transmembrane conductance regulator (CFTR) associated with cystic fibrosis.



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All organisms use an evolutionarily conserved set of molecular machines for the synthesis, folding, transport, and removal of unnecessary and damaged proteins. The PN evolutionarily has adapted to the highly specific physiologic requirements of tissues and the expression of abundant and rare proteins with wide-ranging solubilities, folding requirements, stabilities, and structural demands. Added to this complexity of natural clients for the PN is the additional load generated by genetic mutations carried in natural populations and environmental stressors that challenge the capacity of the PN. Despite the central role of proteins as the workhorse of the cell, they are highly susceptible to molecular damage, whether due to intrinsic metastability or genetically inherited mutation or because of error-prone synthesis. Hence, dysfunction in the PN may clinically manifest as either a gradual decline in homeostatic function as occurs with genetic mutations or a loss of resilience in the face of environmental stressors. Thus, clinicians see the consequences of proteostasis failure and cellular dysfunction in both the myriad disorders that present to them as age-associated clinical problems and the increased morbidity and mortality associated with trauma, infection, and other acute illness requiring hospitalization in older individuals.

PROTEIN QUALITY CONTROL MECHANISMS

The PN monitors and controls the flux of protein synthesis in order to promote functional folding and to minimize the accumulation of off-pathway aggregation-prone intermediates by their selective disaggregation or degradation. However, unlike an automobile assembly plant for which each item is designed and engineered for assembly into functional components, the properties of the PN tolerate the tremendous chemical noise and diversity generated by coding region polymorphisms and biosynthetic errors among its client components, while maintaining the ability to recognize and

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remove kinetically unstable conformational states of proteins that would compromise assembly and function. Proteins are highly sensitive to fluctuations in their intracellular environment caused by shifts in energetics, pH, oxidizing and reducing conditions, and the myriad of small molecules and metabolites that affect folding and function. Added to this range of perturbations are the effects of external stress caused by elevated temperatures, infections, abnormal redox environments, or osmolytes that can have profound consequences on protein-folding thermodynamics, kinetics, and function. These intracellular and extracellular stress conditions, if not properly addressed, would be predicted to amplify further protein instability from sequence polymorphisms and biosynthetic errors that contribute to the stress of protein misfolding. The PN is organized at the cellular level into a series of highly coordinated molecular machines that direct the expression, biogenesis, and functional health of essentially all proteins (Fig. 491-2). Serving more than as regulator and orchestrator of these highly synchronized events, the PN is essential for protein quality control and prevention of the appearance of off-pathway conformational states and accumulation of aggregates and amyloid species. Proteome health involves the constant exchange between the intrinsic physicochemical properties of polypeptides and the biological milieu of the cellular environment in which protein sequences and function evolved.

Beginning with the synthesis of the nascent polypeptide on the ribosome, ribosome quality control (RQC) factors and cytoplasmic chaperones of the HSP70, HSP90, DNAJ/HSP40, chaperonin/HSP60, and small heat shock proteins (sHSPs) family ensure co-translational and posttranslational folding for the cell. Approximately 60% of the proteome resides in the cytoplasm and nucleus, for which the RQC, HSP70, HSP90, and HSP60 chaperones regulate the folded state of client proteins, together with co-chaperones, through cycles of ATP binding and hydrolysis. Chaperones of the HSP70 and J-domain family are particularly well studied for their ability to interact transiently with nascent polypeptides in protein synthesis through short, dispersed, hydrophobic regions using the energy from nucleotide hydrolysis to stimulate the release of partially folded intermediates that either reenter the chaperone cycle or are in a folded native state. For other clients, such as transcription factors, kinases, phosphatases, and signaling molecules, folding to the functional state is regulated and dependent upon interactions with the HSP90 chaperone and other regulatory co-chaperones to form stable heteromeric complexes to maintain the client in a partially folded state primed for subsequent regulated release.

Consistent with the recognition that the formation of off-pathway aggregates is a kinetic component of proteostasis are the concerted activities of disaggregase machines that can unravel protein aggregates into the unfolded, extended polypeptide chain. These disaggregases correspond to the AAA^+ proteins, corresponding to ClpB in bacteria and Hsp104 in yeast and plants and functionally related to a redirected HSP70 machine in other eukaryotes and metazoans that interacts with HSP110 and specific combinations of J-domain proteins capable of inducing disaggregation.

The subcellular organelles, the mitochondria, and endoplasmic reticulum (ER) are responsible for ~20% of the proteome. Chaperone interactions are essential to maintain the extended polypeptide chain in a recognition-competent state for the respective organellar receptors required for translocation across membranes. Upon import, each translocated polypeptide is met by organellar-specific chaperones of the HSP70 and J-domain family for folding and assembly. While the mitochondrial genome encodes 13 proteins required for electron transport, the great majority of mitochondrial proteins are encoded by the nuclear genome, synthesized in the cytosol, and then imported across the outer and inner mitochondrial membranes. Hence, maintenance of the mitochondrial proteome relies on both the cytosolic and mitochondrial PN. For translocation into the lumen of the ER, the extended polypeptide interacts with a set of glycosyltransferases, calnexins, calreticulins and disulphide isomerases. Proteins that misfold in the ER are recognized and retrotranslocated to the cytoplasm, where they are directed to the ubiquitin-proteasome system (UPS) for unfolding and degradation.

The PN is balanced by the essential catabolic processes of the ubiquitin-proteasome and the autophagy-lysosomal machines that recognize proteins for degradation and recycling. The UPS is generally considered the primary pathway by which most proteins are recognized and tagged for degradation; the autophagy-lysosome system is highly responsive to nutrient changes and damage, recognizing and engulfing organelles and other subcellular compartments and large aggregates and inclusions. In addition to their role in the regulated turnover of cellular proteins, these degradation systems are essential for protein quality control and for limiting the accumulation of misfolded and aggregated proteins during stress conditions, aging, and disease.

Protein turnover by the UPS involves an enzymatic cascade of E1, E2, and E3 enzymes that utilize ubiquitin to tag clients, followed by degradation of the polyubiquitinated substrates by the 26S proteasome. Client specificity involves the large family of ~600 ubiquitin ligases. In addition to their roles to mark proteins for degradation, the ubiquitination machinery has numerous additional functions in cellular processes. For example, the ubiquitin ligase listerin is associated with the ribosome and ubiquitinates nascent chains that stall in translation to prevent the accumulation of aberrant polypeptides that would subsequently aggregate. Ubiquitination of these nonnative clients by the ubiquitin ligase activity of the co-chaperone CHIP is central to the triage decision of the HSP70/HSP90 complex between client folding and proteasome-mediated degradation. ER-targeted clients that are misfolded are retrotranslocated to the cytoplasm, polyubiquitinated, and degraded by cytosolic proteasomes in a process termed *ER-associated*

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degradation (ERAD). Ubiquitination also provides cross-talk between the proteasome and autophagy pathways by targeting clients for lysosomal degradation and for endosomal sorting. Chaperones co-label a protein as nonnative, recruiting other proteins that place ubiquitin chains on the damaged protein for degradation by the proteasome. Alternatively, chaperones can label proteins or protein aggregates to target them to the lysosome. In this process, damaged proteins are degraded by the lysosome, an intracellular organelle with an acidic environment enriched in proteases, through autophagy.

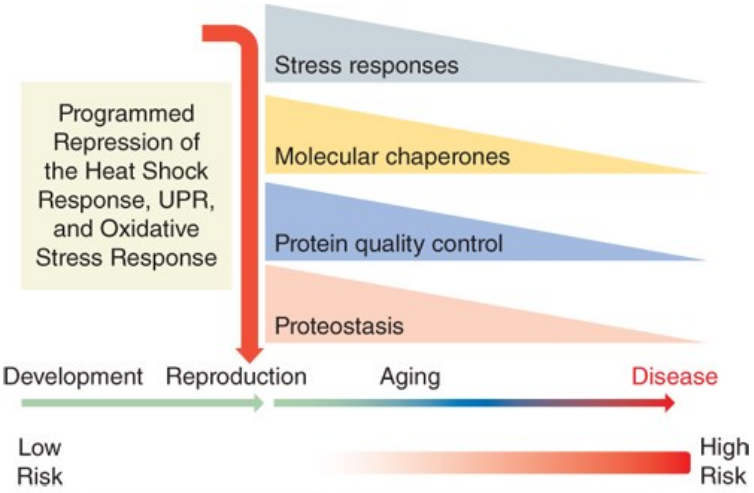
While there is a good general understanding of the process of in vivo chaperone-dependent protein folding, the details of how decisions are made for each client in the cell—whether and for how long to be maintained in a nonnative folding state through chaperone interactions in a nucleotide-independent state, or to assemble into a stable chaperone complex for subsequent assembly into a functional state, or to interact with chaperones to fold directly to a native state—remain to be fully addressed.

CELL STRESS RESPONSES: SENSORS AND REGULATORS OF PROTEIN DAMAGE

Cell stress responses are ancient genetic networks that detect, adapt to, and protect all cells against toxic environmental stressors and physiologically relevant changes in their cellular environment **(Fig. 491-3)**. At the core of these cell stress responses are molecular switches: (1) the heat shock response (HSR) that protects proteins in the cytoplasm and nucleus controlled by HSF-1; (2) the unfolded protein response (UPR) of the ER (UPR^{ER}) controlled by XBP1, ATF6, and ATF4; (3) the UPR of the mitochondria (UPR^{mito}) controlled by ATFS1; (4) the DAF-16/FOX-O stress response pathway associated with insulin signaling; and (5) the antioxidant stress response regulated by NRF-2. Collectively, these cell stress responses and their respective transcription factors are each essential for all cells and tissues and are regulated both autonomously and cell nonautonomously across tissues in metazoans to detect proteotoxic stress and to adapt to and protect the cell against the toxic consequences of protein damage. While each of these cell stress pathways can be activated independently, they are also induced in different combinations according to the chemical and physiologic properties of the stress signal(s) and provide cross-protective mechanisms.

FIGURE 491-3

Aging and protein folding diseases. Aging is the major risk factor for degenerative diseases. Cell stress responses (heat shock response and the unfolded protein responses in the endoplasmic reticulum and mitochondria) decline at reproductive maturity in studies from *Caenorhabditis elegans* and prevent adaptive and protective increased expression of molecular chaperones to prevent protein misfolding. UPR, unfolded protein response.



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The HSR is an evolutionarily conserved cellular defense mechanism that protects cells against proteotoxicity associated with misfolding, aggregation, and proteome mismanagement. HSF-1 inducibly regulates transcription of genes encoding molecular chaperones and components of the PN. In unstressed cells, HSF-1 is cytoplasmic or nuclear and exists in an inert monomeric state negatively regulated by weak interactions with the molecular chaperones HSP70 and HJD-1. Upon heat shock stress, HSF-1 rapidly trimerizes to acquire DNA-binding activity and undergoes extensive posttranslational modifications, binds to heat shock elements in promoters of stress responsive genes, and relocalizes into nuclear stress bodies.

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Upon dissipation of the stress signal, attenuation of the HSR involves the active repression of HSF-1 DNA binding through acetylation and loss of HSF-1 transcriptional activity by reassociation with HSP70 and other molecular chaperones and with HSBP1, leading to dissociation to the monomeric inert state. In addition to HSF-1 being essential for the HSR and cell and organismal stress resilience, HSF-1 is essential during early development in metazoans, functions as a maternal factor for gametogenesis, regulates oocyte maturation by activating genes that function in the meiotic cell cycle, is constitutively activated in cancer, and is necessary to maintain NAD^+ and ATP levels.

In the ER, the UPR^{ER} involves three stress arms regulated by the TFs, XBP1, ATF6, and ATF4, that bind to specific *cis*-elements for these ER-stress-responsive arms. XBP1 is activated by the transmembrane endoribonuclease IRE1, which is a transmembrane protein with kinase and endoribonuclease (RNase) activity that senses misfolding in the ER directly, leading to autophosphorylation, oligomerization, and acquisition of RNase activity. These events allow active IRE1 to cleave *XBP1* mRNA, generating a spliced transcript (*XBP1s*) that encodes XBP1, which induces the transcription of UPR target genes. ER stress also promotes the relocalization of ATF6 from the ER membrane to the Golgi apparatus, where it is cleaved by the proteases SP1 and SP2, generating a cytosolic fragment of ATF6 that translocates to the nucleus to direct transcription of a complementary set of UPR genes. Together, XBP1 and ATF6 induce the expression of genes involved in protein folding, ER-associated protein degradation, and lipid metabolism pathways. A third ER transmembrane protein, PERK, also induced by ER stress, promotes translation of the TF ATF4 by phosphorylating the translation initiation factor eIF2α. Under these conditions, ATF4 mRNA is preferentially translated, leading to selective expression of the proapoptotic TF CHOP, which elicits apoptosis in cells in which ER stress is not resolved, presumably to remove damaged cells from the population.

For mitochondria, the UPR^{mito} response involves ATFS1, which contains a mitochondrial targeting sequence and a nuclear localization signal. Under normal cellular conditions, ATFS1 is imported into mitochondria and degraded, but upon mitochondrial stress, ATFS1 is directed only to the nucleus to regulate transcription of genes encoding mitochondrial chaperones, mitochondrial import machinery, and glycolysis. In mammals, the UPR^{mito} is regulated by ATF5, which corresponds to ATFS-1 in *Caenorhabditis elegans*.

In metazoans, the integration of stress survival strategies includes the antioxidant factor SKN-1/NRF2, the insulin-signaling factor DAF-16/FOXO, and the tissue identity factor PHA-4/FOXA. Oxidative and xenobiotic stresses activate OxR, which controls the expression of redox-regulatory proteins and components of protein degradative pathways mediated in mammals by NRF1/NFE2L1 and NRF2/NFE2L2, which corresponds to SKN-1 in *C. elegans*. NRF1 is an ER-resident factor that undergoes regulated proteolytic cleavage upon activation to control expression of genes encoding subunits of the proteasome and the UPS. NRF2 in the cytoplasm is negatively regulated by the redox-sensitive ubiquitin ligase KEAP-1; consequently, inactivation of KEAP-1 by oxidative and electrophilic stress leads to stabilization and nuclear translocation of NRF2, which, in turn, induces the expression of antioxidant proteins and detoxification enzymes.

ORGANISMAL PROTEOSTASIS IN AGING AND DISEASE

Much of our understanding on protein quality control mechanisms has come from in vitro studies with purified molecular chaperones or components of the UPS, complemented with cell extracts and cell-based assays using yeast or mammalian cells grown in tissue culture. A common theme that emerges from these studies is that of hormesis, in which transient bouts of activation of the HSR, UPR^{ER}, or UPR mito improves lifespan and organismal resilience but chronic activation is detrimental.

The importance of these pathways is further highlighted by studies suggesting neuronal coordination of stress responses at the level of the organism. When neuronal mechanisms fail, the HSR reverts to cell-autonomous control. At the organismal level, however, the HSR, UPR^{ER}, and the UPR mito in *C. elegans* are regulated by cell-nonautonomous control through neuronal signaling. When neuronal signaling is impaired, the HSR reverts to cell-autonomous control. Neuronal signaling also regulates the UPR mito with disruption of mitochondrial function in *C. elegans* neurons activating the UPR mito in nonneuronal tissues, supporting a role for a mitokine signal. Perturbation of the mitochondrial electron transfer chain (ETC) was shown to increase life span in both invertebrates and rodents through the activation of the UPR mito. The response to mitochondrial dysfunction in *C. elegans* depends upon the severity of mitochondrial impairment with a mild reduction of ETC having hormetic effects on organismal stress resilience, proteostasis, and longevity by resetting the cytoplasmic HSR through HSF-1, independent of ATFS-1 and the UPR mito. Mild perturbation of the ETC in *Drosophila* muscle also has systemic benefits on organismal health and life span involving the insulin signaling. Communication between neurons also regulates the UPR^{ER} in peripheral tissues of *C. elegans*. During infection of *C. elegans* by pathogens, induction of the UPR^{ER} in nonneuronal tissues is mediated by sensory neurons, suggesting an organismal stress response. Cell-nonautonomous regulation of the UPR^{ER} has also been observed in mice, where overexpression of active XBP1 in pro-opiomelanocortin neurons activates the UPR^{ER} in the liver.

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Other forms of intertissue communication that regulate proteostasis with beneficial effects on organismal health include: cholinergic signaling across the synaptic junction to enhance or inhibit HSF-1-regulated proteostasis in the muscle cells of *C. elegans;* transcellular chaperone signaling between somatic tissues or between somatic tissues and neurons of *C. elegans* to regulate the expression of HSP90 in receiving tissues via the tissue code factor PHA4/FOXA, protection of neurons and glial cells from elevated temperature-induced death by overexpression of small HSPs in *Drosophila* flight motor muscle cells, enhancing proteostasis in *C. elegans* muscle cells by elevated expression of DAF16/FOXO in the intestine, and that overexpression of dFOXO/4E-BP in *Drosophila* muscle influences proteostasis in retina, brain, and adipose tissues to delay the age-dependent accumulation of protein aggregates.

Cell stress responses and proteostasis decline in aging. Insights on the relationship between proteostasis, cell stress, and aging have come primarily from *C. elegans* with support from other invertebrate and vertebrate model systems and human cells. A set of endogenous metastable proteins that exhibit temperature-sensitive properties were shown to misfold in *C. elegans* at the permissive temperature during aging, which was associated with a decline in the HSR. The decline of proteostasis in *C. elegans* aging is regulated via cell-nonautonomous control involving germline stem cells that initiate the programmed repression of the organismal HSR resulting in the loss of stress resilience and age-associated protein aggregation. This is regulated at reproductive maturity by an epigenetic switch involving the timed placement of repressive H3K27me3 chromatin marks at the heat shock genes, causing chromatin inaccessibility for HSF-1. This age-dependent decline in the HSR can be reversed either by blocking the signal from germline stem cell signal(s) or preventing the epigenetic repressive marks. The relationship between reproduction and inducibility of the HSR observed in animals at reproductive maturity suggests that the age-associated events of cellular failure and loss of tissue robustness during aging are not random processes but, rather, highly regulated, perhaps to ensure that somatic tissues are programmed to decline after reproduction, consistent with the germline soma theory of aging.

Proteostasis is one of the pillars of gerontologic biology, which, together with genomic instability, telomere attrition, epigenetic alterations, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication, provides a mechanistic basis for the biology of aging. The programmed decline of proteostasis in early adulthood would suggest that failure in protein quality control would have negative consequences on the other key elements of gerontologic biology. Whether proteostasis collapse is the first to fail or among the earliest events to fail in aging, it is consistent with the very large number of human degenerative diseases in aging associated with protein misfolding.

PROPERTIES OF PROTEIN FOLDING DISEASES

The complexity that arises with protein folding diseases is that all tissues are at risk and essentially all proteins are at risk for misfolding. Added to this is the effect of aging and that each protein folding disease exhibits a highly variable age of onset for pathology. There is additional complexity in classification—whether to organize folding diseases by tissues, i.e., muscle proteinopathies or neurodegenerative diseases, according to the specific protein that misfolds such as α_1 antitrypsin (AAT) deficiency, or by the biophysical nature of the aggregate species in systemic amyloidoses.

Disorders in which a specific mutation leads to protein misfolding or the formation of a specific insoluble protein aggregates likely represent only the tip of the iceberg of protein folding disorders. Mutations in aggregation-prone proteins coupled with changes in the cellular environment and decline in capacity and robustness of the PN in aging promote protein misfolding and aggregation in affected tissues. Once aggregation has initiated, this leads to further impairment in protein quality control pathways, causing further collateral damage and aggregation of other at-risk proteins. Such a mechanism may only manifest clinically after a seemingly random systemic stress like pneumonia, large bone fractures, or ischemic vascular events, possibly explaining the rapid (1–2 years) accumulation of age-related morbidities in the year following the first event. As such, the age-related decline in the function of any of the components of the PN could underlie the compounding multiple morbidity that limits health span and life span in many elderly individuals. Within this framework, it is useful to discuss some of the better understood mechanisms of proteostasis dysfunction that have been causally linked to diseases in humans.

DISORDERS THAT ENHANCE MISFOLDING AND CAUSE PREMATURE DEGRADATION (CYSTIC FIBROSIS)

Cystic fibrosis (CF) is a recessive disorder caused by mutations in both alleles of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that encodes a multidomain membrane-spanning chloride ion channel protein. Thousands of mutations in *CFTR* have been identified that affect *CFTR* biosynthesis, folding, trafficking, and function, leading to chronic obstructive lung disease, intestinal obstruction, liver dysfunction, exocrine and endocrine pancreatic dysfunction, and male infertility. CF is a folding disease due to its recognition by the PN as misfolded protein. The most

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prominent mutation is deletion of phenylalanine 508 (F508del), present in ~90% of CF patients. Mutant ΔF508 retains partial channel function, but because it is recognized as misfolded in the ER and the cytoplasm, it is marked with ubiquitin for degradation by the ubiquitin proteasome system. Small molecules that affect the conformation and function of mutant ΔF508 channel function can result in substantially improved outcomes in patients.

DISORDERS THAT INDUCE TOXIC AGGREGATES AND LOSS OF FUNCTION (AAT DEFICIENCY)

AAT deficiency (AATD) is a co-dominant inherited disease with an increased risk of chronic obstructive pulmonary disease (COPD), liver disease, and vascular inflammation. Pulmonary problems are more frequent in adults, whereas liver and skin problems may occur in adults and children. AAT is encoded by the *SERPINA1* gene, secreted into the circulation by the liver, and responsible for inactivating endogenous proteases, particularly those secreted by neutrophils and other inflammatory cells in the lung. Patients with AATD harbor mutations in *SERPINA1* that cause misfolding in the ER. The two major phenotypes resulting from this abnormality highlight the diverse consequences of misfolding on different cells and organs. In the liver, misfolding of the mutant protein results in the formation of toxic aggregates and hepatocyte death, manifest as liver injury and eventually cirrhosis—a gain-of-function toxicity. In the lung, the failure to secrete sufficient AAT causes unchecked proteolytic damage to the delicate architecture of the alveolus, a process that is markedly worsened when neutrophils are recruited to the lung in response to cigarette smoking. This loss-of-function phenotype manifests pathologically as emphysema and clinically as COPD.

INTERACTIONS WITH PN COMPONENTS THAT CHANGE CONFORMATION, STABILITY, OR FUNCTION (CANCER)

Mutations in the tumor suppressor p53 are among the most common mutations observed in patients with cancer. Deletion of p53 combined with overexpression of an oncogene is sufficient to drive metastatic cancer formation in mice, causally linking p53 mutations with cancer. Normally, p53 functions as a transcription factor that suppresses the transcription of genes involved in apoptosis resistance. While myriad mutations in p53 have been described, some result in an alternate conformation that interacts with different HSP70 chaperones within the PN. Binding of the mutant p53 protein to these chaperones affects the DNA binding property necessary for its tumor-suppressor function and facilitates binding to other domains, resulting in changes in gene expression that protect malignant cells from apoptosis.

STRONGLY ENHANCED AGGREGATION PROPENSITY AND AMYLOID FORMATION (ALZHEIMER'S DISEASE, PARKINSON'S DISEASE, AMYOTROPHIC LATERAL SCLEROSIS, HUNTINGTON'S DISEASE, TYPE 2 DIABETES MELLITUS)

In some individuals, native or mutant proteins include sequence motifs that promote an alternate highly ordered aggregation state when the cellular environment is altered and in aging. The most common of these motifs are the beta-pleated sheets, which, when exposed to the solvent environment of the cell, easily bind to one another in an iterative process that can accommodate many thousands of molecules that form insoluble cross-beta sheet amyloid species. Broadly, these intracellular aggregates are classified as oligomers (2–24 molecules), protofibrils (rods 4–11 nm wide and 200 nm long), and amyloid fibrils with a similar width to protofibrils but microns in length. While the formation of oligomers is thermodynamically unfavorable, polymerization is favorable, causing aggregates to seed slowly but grow exponentially. In some cases, for example, Huntington's disease, familial forms of Alzheimer's disease, and ALS, aggregation is accelerated by mutations. However, in many cases, the aggregates contain other cellular proteins that share biophysical properties of aggregation propensity or reflect dysfunction in the PN that facilitates their seeding or propagation (see below). While in most instances, damage caused by protein aggregates is localized to the cells in which they form, as occurs with islet amyloid peptide in some patients with type 2 diabetes, amyloidogenic proteins associated with neurodegenerative diseases have been shown to "spread" between cells. Damage to neurons by aggregates in Alzheimer's disease can elicit a local inflammatory response by resident immune cells in the brain, both of which contribute to pathology. Much effort has been directed toward the detection of aggregates and amyloid and the development of small molecules or antibodies that block further growth or the enhancement of cellular activities of the PN to suppress protein misfolding.

SECRETED AGGREGATED AND AMYLOID SPECIES CAUSING SYSTEMIC AMYLOIDOSIS

In patients with systemic amyloidosis, the secretion of large amounts of aggregation-prone proteins results in the deposition of aggregates in many tissues. These proteins can include immunoglobulins secreted from plasma cells in patients with systemic inflammation or multiple myeloma or other aggregation-prone proteins including transthyretin. Similar to other aggregate-induced diseases, mutations in transthyretin that facilitate polymerization are associated with an increased risk of developing systemic amyloidosis with advancing age. These aggregates induce cellular toxicity, inflammation, and matrix reorganization that interfere with function in an organ-specific manner. Deposition of amyloid produces stiffness where

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there should be flexibility, creates barriers where there should be free flow, and distorts size where there should be fit. The stiffness is particularly damaging to the heart, lung, and blood vessels and both smooth and skeletal muscle. The barrier effect can result in malabsorption in the gastrointestinal tract and glomerular dysfunction, cardiac and peripheral nerve conduction defects, and limitation of joint range of motion.

NATIVE PROTEINS PRONE TO AGGREGATE WHEN THE CELLULAR ENVIRONMENT IS ALTERED BY STRESS AND AGING

While well-defined genetic abnormalities have been essential in elucidating the molecular mechanisms that underlie the formation of protein aggregates and causally linking them to disease, many, if not most, clinical diseases associated with the formation of protein aggregates develop in patients without identified mutations. In these patients, a decline in the chaperone and quality control mechanisms of the PN allows exposure of aggregation-prone domains of normal proteins to the solvent environment of the cell. Once seeded, these protein aggregates can expand rapidly to induce local or systemic injury. The decline in function of the PN that allows these aggregates to form might develop gradually with advancing age or might occur suddenly in response to an age-triggered biologic program, as occurs in *C. elegans*.

INFECTIOUS DISEASES AND IMBALANCED CELL STRESS RESPONSES IN AGING

A model in which the function of the PN is reduced in aging might explain the disproportionate morbidity and mortality in older individuals exposed to systemic stress. While these stressors include infections, surgical or accidental trauma, sepsis, and myocardial infarction, among others, pneumonia, the most common cause of death from an infectious disease in the United States, provides an illustrative example. As was evident during the COVID-19 pandemic, pneumonia morbidity and mortality disproportionately affect the elderly. Viral pneumonias, including those caused by influenza viruses and SAR-CoV-2, are primarily localized to the lung, where they activate a local and systemic inflammatory response and denude the alveolar lining. The resulting hypoxemia and systemic inflammatory response injures distant organs independent of viral injury. Impaired function of the PN during the stress might allow seeding of tissues with toxic aggregates with long-term consequences. Repair of the damaged lung and distant organs represents a major challenge to proteostasis that might be overcome in younger individuals but fail in those who are older with poor stress resilience. This loss of proteostasis resilience necessary to limit damage and allow repair could explain clinical observations in pneumonia survivors who develop persistent lung injury, skeletal muscle dysfunction impairing mobility, chronic kidney disease, cognitive dysfunction and dementia, and an increased risk of ischemic cardiovascular events in the year after hospital discharge.

FURTHER READING

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