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Protein Folding Disorders Many hundreds of human diseases, collectively known as protein con formational diseases or protein folding disorders, result from protein misfolding due to intrinsic and extrinsic errors amplified by aging and exposures to environmental and physiologic stress conditions. Such events challenge the functional integrity of the proteome and can lead to dysfunction, enhanced aggregation of proteins, mislocalization, and premature or inhibition of protein clearance, thus affecting cellular robustness, health, and longevity. Mismanagement of the proteome is therefore the basis of a broad class of diseases that includes orphan lysosomal storage diseases, type 2 diabetes mellitus, cystic fibrosis, some fibrotic diseases, metabolic diseases, muscle-wasting diseases, cancer, and neurodegenerative diseases exemplified by Alzheimer’s disease, frontal temporal dementia, Parkinson’s disease, amyotrophic lateral sclerosis (ALS), and Huntington’s disease (Fig. 504-1). For each of these diseases and many others described in this textbook, aging is the major contributing risk factor. The challenge at the biochemical and molecular level is for the cell to achieve and maintain a stable and functional proteome during development that persists through young adulthood and is maintained throughout aging. For humans, this is essential for the operational health of each of the tens of trillions of cells that comprise our ~80 organs for health span and lifespan. To achieve this, our cells have evolved a remarkably efficient proteostasis network (PN) composed of ~3000 molecular chaperones and other highly conserved components essential for protein synthesis, folding, translocation, and degradation (Fig. 504-2) that balances input with output and ensures that every protein is functional. The PN is, therefore, essential for the robust ness of all tissues and for the diverse protein-protein interactions in cell signaling, biosynthetic processes, and the structural demands and mechanical requirements for tissue shape and function. An equal, if not more important, role for the PN is to detect, prevent, and remove PART 20 Emerging Topics in Clinical Medicine Eye Neuronal tissue Cataracts Corneal lactoferrin amyloidosis Hereditary lattice corneal dystrophy Lung Pulmonary alveolar proteinosis Cystic fibrosis Muscle Inclusion body myositis/myopathy Aortic medial amyloidosis Cardiac amyloidosis (e.g., transthyretin cardiomyopathy) FIGURE 504-1 Diseases of protein folding. A representative list of tissues affected and known folding diseases.

misfolded and aggregated proteins that accumulate in stress, aging, and disease and interfere with cellular health. Understanding how proteo stasis is achieved and maintained is of fundamental

biological interest and essential to prevent age-associated protein folding disorders. Con sequently, there is tremendous interest in the detection and treatment of these diseases, in particular as the human population continues to live longer. All organisms express an evolutionarily conserved set of molecular machines for the synthesis, folding, transport, and removal of unneces sary and damaged proteins. The PN is adapted for the highly specific physiologic requirements of tissues and the expression of abundant and rare proteins with wide-ranging solubilities, folding requirements, stability, 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 together with diverse environmental stressors that challenge PN capacity. Despite the central role of proteins as the workhorse of the cell, they are also highly susceptible to molecu lar damage, whether due to intrinsic metastability or to genetically inherited mutation or error-prone synthesis. Hence, dysfunction in the PN may clinically manifest as a gradual decline in homeostatic func tion in aging as occurs with genetic mutations or a loss of resilience in the face of environmental stressors. Thus, clinicians see the con sequences of proteostasis failure and cellular dysfunction in both the myriad disorders that present to physicians 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 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 part is designed and engineered for a specific use and precise assembly, the PN has built-in functional redundancy with properties to tolerate tremendous chemical noise and sequence diversity gener ated by coding region polymorphisms and biosynthetic errors among its client components. The PN has the ability to recognize and remove kinetically unstable conformational states of pro teins that accumulate in aging and that would otherwise compromise assembly and function. Proteins are highly sensi tive to fluctuations in their intracellular environment caused by shifts in ener getics, pH, and oxidizing and reducing conditions in addition to the myriad small molecules and metabolites that affect folding and function. Added to changes are the effects of external stress conditions caused by elevated tempera tures, infection, oxidizing and reducing environments, or osmolytes that can have profound consequences on pro tein folding thermodynamics, kinetics, and function. These intracellular and extracellular stress conditions, if not properly responded to, are predicted to further amplify protein instability from sequence polymorphisms and biosyn thetic errors that contribute to the stress of protein misfolding. The PN is orga nized at the cellular level into a series of highly coordinated molecular machines that direct the expression, biogenesis, and functional health of essentially all Alzheimer’s disease Amyotrophic lateral sclerosis Familial British dementia Familial Danish dementia Parkinson’s diease Huntington’s disease Thyroid Medullary thyroid carcinoma Immune system Systemic AL amyloidosis Multiple myeloma Pancreas/islet a cells Type 2 diabetes mellitus Liver α1 Antitrypsin deficiency Systemic diseases Lysozyme storage diseases p53-dependent cancers

Molecular chaperones Unfolded nascent polypeptide Native state Intermediate folded states Chaperones Autophagy Proteasome Normal turnover Misfolded states Degradation Toxic folds Improper trafficking Cystic Fibrosis Amyloidoses Emphysema Abeta, tau, Huntington, SOD1, α-synuclein α1 Antitrypsin Cystic fibrosis transmembrane conductance regulator FIGURE 504-2 The proteostasis network (PN) and folding diseases. Protein biogenesis through the action of molecular chaperones ensures the transition of the nascent polypeptide to on-pathway intermediates and the

folded functional native state. Such proteins then have a normal turnover mostly through the ubiquitin-proteasome system. Off-pathway species are prevented by the actions of chaperones and the recognition of nonnative misfolded states by the autophagiclysosomal pathway and the ubiquitin-proteasome system. When misfolded species escape quality control or overwhelm the PN, they can then become improperly trafficked as occurs for α1 antitrypsin associated with emphysema; for toxic folds as occurs for abeta, tau, Huntington, and SOD1 in amyloidogenic neurodegenerative diseases; and prematurely degraded as occurs for CFTR associated with cystic fibrosis. proteins (Fig. 504-2). More than to regulate and orchestrate these highly synchronized events, the PN is essential for protein quality control and for the prevention of the appearance of off-pathway con formational states and condensates, with 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) together with cytoplasmic chaperones of the HSP70, HSP90, DNAJ/HSP40, chaperonin/HSP60, and small HSPs (sHSPs) family ensure co- and posttranslational fold ing for the cell. Approximately 60% of the proteome resides in the cytoplasm and nucleus, for which the RQC, HSP70, HSP90, and HSP60 chaperones together with co-chaperones regulate the folded state of cli ent proteins 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 short dispersed hydrophobic regions of nascent polypeptides using the energy from nucleotide hydrolysis to regulate the release of partially folded intermediates that either reenter the chaperone cycle or are released in a folded native state. For other chaperone clients, such as transcription factors, kinases, phosphatases, and signaling molecules, their folding to the functional state is highly regulated and dependent upon interactions with the HSP90 chaperone and other regulatory co-chaperones to form stable heteromeric complexes that hold 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 chaperone machines with disaggregase activity that unravel protein aggregates for refolding. These disaggregases include the AAA+ protein, ClpB in bacteria, Hsp104 in yeast and plants, and the function ally analogous metazoan disaggregase composed of HSP70, HSP110, and specific J-domain proteins. The subcellular organelles mitochondria and endoplasmic reticu lum (ER) account for ~20% of the proteome. Chaperone interactions are essential for mitochondrial-targeted proteins to maintain the extended polypeptide chain in a recognition-competent state for the

organellar receptors 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 trans port, the great majority of mitochondrial proteins are encoded by the nuclear genome, synthesized in the cytosol, and imported across the outer and inner mitochondrial membrane. Hence, maintenance of the mitochondrial proteome relies on the coordinated efforts of both the cytosolic and mitochondrial PN. For translocation into the lumen of the ER, the extended polypeptide interacts with a set of glycosyl transferases, calnexins, calreticulins, disulphide isomerases, and lumen localized chaperones. Proteins that misfold in the ER are recognized and retro-translocated to the cytoplasm where they are directed to the ubiquitin-proteasome system (UPS) for unfolding, ubiquitination, and degradation.

The PN is balanced by the essential catabolic processes of the UPS and the autophagy-lysosomal pathway (ALP), which recognizes pro teins for degradation and recycling. The UPS is generally considered the primary pathway by which most proteins are recognized and tagged for degradation, and the ALP is highly responsive to nutrient deprivation and damage to recognize large aggregates and inclusions and engulf organelles and other subcellular compartments. In addition to their role in the regulated turnover of cellular proteins, these degra dation 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 and the recognition selectivity of E3s to tag clients, followed by degrada tion of the polyubiquitinated substrates by the 26S proteasome in the cytoplasm. Client specificity involves the large family of ~750 ubiquitin E3 ligases. In addition to their role of marking proteins for degradation, the ubiquitination machinery has numerous additional functions in cellular processes. For example, the ubiquitin ligase listerin is associ ated with the ribosome to ubiquitinate nascent chains that stall during protein synthesis to prevent the accumulation of aberrant polypep tides that would subsequently aggregate. Ubiquitination of nonnative aggregated clients by the ubiquitin ligase activity of the cochaperone carboxyl terminus Hsc70 interacting protein (CHIP) is central to the triage decision of the HSP70/HSP90 complex between client fold ing and proteasome-mediated degradation. ER-targeted clients that are misfolded are retro-translocated to the cytoplasm where they are polyubiquitinated and degraded by cytosolic proteasomes in a process termed ER-associated degradation (ERAD). Ubiquitination also pro vides crosstalk between the proteasome and autophagy pathways by targeting clients for lysosomal degradation and for endosomal sorting. Chaperones co-label a protein as damaged, 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, and in this process, damaged proteins are degraded by the lysosome, an intracellular organelle with an acidic environment enriched in proteases through autophagy. CHAPTER 504 Protein Folding Disorders While there is a comprehensive understanding of the process of in vivo chaperone-dependent protein folding, the details of how these decisions are made for each client, whether and for how long to be maintained in a nonnative folding state through chaperone interactions in a nucleotide-independent state, or how to assemble into a stable chaperone complex for subsequent assembly into a functional state or to interact with chaperones to directly fold 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, and protect all cells against toxic environmental stressors and physiologi cally relevant changes in their cellular environment, including changes induced during development and tissue repair after injury (Fig. 504-3). 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 regulated by HSF-1; (2) the unfolded protein response (UPR)

Stress responses Programmed Repression of the Heat Shock Response, UPR, and Oxidative Stress Response Molecular chaperones Protein quality control Proteostasis Disease Aging Reproduction Development High Risk Low Risk FIGURE 504-3 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 [UPR] 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. of the ER (UPRER)

controlled by XBP1, ATF6, and ATF4; (3) the UPR of the mitochondria (UPRmt) controlled by ATFS1; (4) the DAF-16/ FOX-O stress response pathway associated with insulin signaling; (5) the integrated stress response (ISR) controlled by PERK, PKR, HRI, GCN2, and ATF4; and (6) the antioxidant stress response regulated by NRF-2. Collectively, these cell stress responses and their respective transcription factors (TFs) are essential for all cells and tissues regu lated both autonomously and cell nonautonomously across tissues in metazoans to detect proteotoxic stress, to adapt and protect the cell against the toxic consequences of protein damage, and to regulate changes in the proteome necessary for differentiation. 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 crossprotective mechanisms. PART 20 Emerging Topics in Clinical Medicine The HSR is an evolutionarily conserved cellular defense mech anism that protects cells against proteotoxicity associated with misfolding, aggregation, and proteome mismanagement. HSF-1 inducibly regulates the transcription of genes encoding molecular chaperones and components of the PN. In unstressed cells, HSF-1 exists in an inert monomeric state in the cytoplasm or nucleus where it is negatively regulated by the molecular chaperones, HSP70 and HJD-1. Upon exposure to heat shock, HSF-1 undergoes a series of molecular transformations and rapidly trimerizes to acquire DNAbinding activity, undergoes extensive posttranslational modifications by phosphorylation and sumoylation, binds to heat shock elements in promoters of heat shock responsive genes, and associated with these events, forms nuclear stress bodies. Upon dissipation of the stress signal, the HSR attenuates by the active repression of HSF-1 DNA binding by acetylation and loss of HSF-1 transcriptional activity. This is accomplished by binding of HSF-1 with HSP70, HDJ-1, and HSBP1, leading to its dissociation from the trimer to the monomeric state. In addition to HSF-1 being essential for the HSR and cell and organismal stress resilience, HSF-1 is essential during early develop ment 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 neces sary to maintain NAD+ and ATP levels. In the ER, the UPRER involves three stress response arms regulated by the transcription factors XBP1, ATF6, and ATF4 that bind to specific cis- elements for these ER-stress-responsive pathways. XBP1 is activated by IRE1, which is a transmembrane protein with kinase and endoribonu clease (RNase) activity that senses misfolding in the ER directly, leading to its autophosphorylation, oligomerization, and acquisition of RNase activity. This allows active IRE1 to cleave XBP1 mRNA, generating a spliced transcript (XBP1s) that encodes XBP1 to induce 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. A third ER transmembrane protein, PERK, also induced by ER stress, phosphorylates the translation initiation factor eIF2α, linking activation of the UPRER with the ISR. In the mitochondria, the UPRmt response involves ATFS1, which contains a mitochondrial targeting sequence and a nuclear localiza tion signal. Under normal cellular conditions, ATFS1 is imported into mitochondria and degraded, but upon mitochondria stress, ATFS1 is directed only to the nucleus to regulate transcription of genes encod ing mitochondrial chaperones, mitochondrial import machinery, and glycolysis. In mammals, the UPRmt is regulated by ATF5, which is the orthologue of ATFS1 in Caenorhabditis elegans. Inhibitors

of mito chondrial electron transport in mammals also activate the ISR through the release of a protease OMA1 that cleaves a cytosolic protein DELE1 to activate HRI. The ISR is induced by one or more of four kinases: PKR, PERK, HRI, or GCN2. All four of these kinases phosphorylate eIF2a, a key protein in the ternary complex that regulates protein synthesis. The resulting global inhibition of protein synthesis paradoxically pro motes translation of mRNA molecules with specific sequences in their upstream open reading frames. These include the transcription factor ATF4, which induces the expression of a gene program that maintains metabolism to preserve stress resilience. Activation of ATF4 also induces its expression and the expression of Ddit3 (CHOP), lowering the threshold for apoptosis, and Gadd45a, a phosphatase that dephos phorylates eIF2a to restore translation. The ISR is linked to ER stress through PERK and to mitochondrial stress through HRI. GCN2 is activated by amino acid deprivation, and PKR is activated during viral infection. Of particular note has been the development of ISRIB, a small molecule that partially inhibits the activity of the ISR with salu tary effects across diverse animal models of age-related degenerative diseases. These findings suggest that cell stress pathways that are pro teome protective in youth might become pathologic in aging, making them attractive targets for therapeutic intervention. Indeed, activation of the ISR has been shown to impair stem cell differentiation, perhaps linking mitochondrial dysfunction with aging, proteostasis, and stem cell dysfunction. In metazoans, the integration of stress survival strategies includes the antioxidant factor SKN-1/NRF2, the insulin-signaling factor DAF16/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 degrada tive pathways mediated in mammals by NRF1/NFE2L1 and NRF2/ NFE2L2, which corresponds to SKN-1 in C. elegans. NRF1 is an ERresident factor that undergoes regulated proteolytic cleavage upon activation to control expression of genes encoding subunits of the pro teasome 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 expres sion of antioxidant proteins and detoxification enzymes. ORGANISMAL PROTEOSTASIS IN

AGING AND DISEASE Much of our understanding of protein quality control mechanisms has come from in vitro studies with purified molecular chaperones or com ponents of the UPS, complemented with cell extracts and cell-based assays using yeast or mammalian tissue culture cells. A common theme that emerges from these studies is that of hormesis, in which chronic low-level activation of the HSR, UPRER, and UPRmt is protective against subsequent exposures to extreme and lethal cell stress conditions. The importance of these pathways is further highlighted by studies in metazoans that indicate that cell stress responses are regulated at the organismal level by neuronal signaling. At the organismal level in

C. elegans, the HSR, UPRER, and UPRmt are regulated by cell-nonauton omous control by specific sensory neurons. When neuronal signaling is impaired, the HSR reverts to cell-autonomous control. Likewise, neu ronal signaling regulates the UPRmt with disruption of mitochondrial function in C. elegans neurons activating the UPRmt in nonneuronal tis sues, supporting a role for a mitokine signal. Perturbation of the mito chondrial electron transfer chain (ETC) was shown to increase lifespan in both invertebrates and rodents through the activation of the UPRmt. The response to mitochondrial dysfunction in C. elegans depends on the severity of mitochondrial impairment, with a mild reduction of ETC or reduction of mitochondrial proteostasis having hormetic effects on organismal stress resilience, proteostasis, and longevity by resetting the cytoplasmic HSR through

HSF-1, independent of ATFS-1 and the UPRmt. Mild perturbation of the ETC in Drosophila muscle also has systemic benefits on organismal health and lifespan involving the insulin signaling pathway. Communication between neurons also regulates the UPRER in peripheral tissues of C. elegans. During infection of C. elegans by pathogens, induction of the UPRER in nonneuronal tissues is mediated by sensory neurons, suggesting an organismal stress response. Cell-nonautonomous regulation of the UPRER has also been observed in mice, where overexpression of active XBP1 in pro-opiomelanocortin neurons activates the UPRER in the liver. Several other forms of intertissue communication regulate proteos tasis with beneficial effects on organismal health in model organisms. For example, muscle cell proteostasis in C. elegans is regulated by cho linergic signaling across the synaptic junction through modulation of HSF-1 activity. Transcellular chaperone signaling between somatic tis sues, and between somatic tissues and neurons, of C. elegans communi cate proteotoxic stress signals via the tissue code factor PHA-4/FOXA to control systemic expression of HSP90. In Drosophila, overexpression of small HSPs only in the flight motor muscle cells protects neurons and glial cells from elevated temperature-induced death. Enhanced expression of DAF-16/FOXO in the intestine enhances proteostasis in distant muscle cells of C. elegans, and likewise, 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 with insights on the relationships between processes coming primarily from studies using C. elegans with support from other invertebrate and vertebrate model systems and human cells. Endogenous metastable proteins that harbor temperature-sensitive properties misfold in C. elegans at the permissive temperature in early aging associated with a decline in the HSR. This functional decline of proteostasis in C. elegans aging is regulated by cell-nonautonomous control, from the germline stem cells to the somatic tissues for the programmed repression of the organismal HSR, resulting in the loss of stress resilience and proteostasis caus ing age-associated protein aggregation. This HSR repressive switch is regulated by signaling from the germline stem cells to the somatic tissues, resulting in the timed placement of repressive H3K27me3 chromatin marks at the promoters of heat shock genes, causing chro matin 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 rela tionship 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 represents one of the primary hallmarks of the biology of aging, which together with genomic instability, telomere attrition, epi genetic alterations, deregulated nutrient sensing, mitochondrial dysfunc tion, cellular senescence, stem cell exhaustion, and altered intercellular communication provides a mechanistic basis for the aging process. The programmed decline of proteostasis in early adulthood would support the hypothesis that failure in protein quality control would have negative consequences on the other pillars of geroscience. Whether proteostasis

collapse is the first to fail or among the earliest events that fail in aging, it is consistent with 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 all proteins are at risk for misfolding and lossof-function or gain-of-function proteotoxicity. 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 regarding whether to organize folding diseases by tissues (i.e., muscle proteinopathies or neurodegenerative diseases), according to the specific protein that misfolds such as α1-antitrypsin deficiency, or by the biophysical nature of the misfolded or aggregated species in amyloidoses. Disorders in which a specific mutation leads to protein misfolding or the formation of a specific insoluble protein aggregate likely repre sent only the tip of the iceberg of protein folding disorders. Mutations in aggregation-prone proteins coupled with changes in the cellular environment and effects on the capacity of the PN will promote mis folding and aggregation in affected tissues. Chronic stress may cause the aberrant cell stress responses and protein quality control pathways, causing further collateral damage and aggregation of other at-risk proteins. Such a mechanism may only manifest clinically after a seem ingly random systemic stress like pneumonia, large bone fracture, or ischemic vascular event, possibly explaining the rapid (1–2 years) accumulation of age-related morbidities in the year following a major biologic stress, marked by a need for hospitalization. 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 lifespan 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. CHAPTER 504 Protein Folding Disorders ■ ■DISORDERS THAT ENHANCE CLIENT 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 chlo ride ion channel protein. Thousands of mutations in CFTR have been identified that affect CFTR biosynthesis, folding, trafficking, and func tion, 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 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 UPS. Combinations of small molecules that protect the misfolded CFTR protein from degradation and enhance its func tion have led to substantial improved outcomes in many patients with CF (Chap. 302). ■ ■DISORDERS THAT INDUCE TOXIC AGGREGATES AND LOSS OF FUNCTION IN MULTIPLE TISSUES (`1-ANTITRYPSIN DEFICIENCY) α1-Antitrypsin deficiency (AATD) is a co-dominant inherited disease with an increased risk of chronic obstructive pulmonary disease, liver disease, and inflammation of the blood vessels. Pulmonary problems are more frequent in adults, whereas liver and skin problems may occur in adults and children. α1-Antitrypsin is encoded by the SERPINA1 gene and secreted into the circulation by the liver and is 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 fail ure to secrete

sufficient α1 antitrypsin may lead to unchecked proteo lytic 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 mani fests pathologically as emphysema and clinically as chronic obstructive pulmonary disease.

■ ■INTERACTIONS WITH PN COMPONENTS

THAT CHANGE CONFORMATION, STABILITY,

OR FUNCTION (CANCER) Mutations in the tumor suppressor p53 are among the most com mon 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 chap erones 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, result ing 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 a highly ordered aggregation state when the cellular environment is altered. The most common of these motifs is the beta sheet, which, when exposed to the solvent environment of the cell, forms intermolecular species that bind in an iterative process that can accommodate many thousands of molecules that form cross-beta sheet amyloids. These intracellular aggregates are described 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 oligo mers is thermodynamically unfavorable, polymerization is favorable, causing aggregates to seed slowly but grow exponentially. In some cases, for example in Huntington’s disease and familial forms of Alzheimer’s disease and ALS, aggregation is accelerated by mutations or expansion of homopolymers. However, in many cases, the aggre gates contain other cellular proteins that share biophysical proper ties 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 mellitus, amyloidogenic proteins asso ciated with neurodegenerative diseases have been shown to spread between cells and, in the case of transthyretin amyloidosis, can cause pathology in many tissues. Pathologists use staining of tissues with Congo red, which detects beta sheets, to make this diagnosis. Dam age 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 enhancing the cellular activities of the PN to suppress protein misfolding. PART 20 Emerging Topics in Clinical Medicine ■ ■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 enhance polymerization are associated with an increased risk of developing systemic amyloidosis with advancing age. These aggregates induce cellular toxicity, inflammation, and matrix reorganization, which interfere with function in an organ-specific manner. ■ ■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 The response to infectious diseases and the disproportionate morbidity and mortality in older individuals exposed to systemic stress are likely associated with the decline in robustness of cell stress responses and proteostasis. While these stressors include infections, surgical or acci dental trauma, sepsis, and myocardial infarction, among others, pneu monia, 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 mortal ity disproportionately affect the elderly. Viral pneumonias, including those caused by influenza viruses and SAR-CoV-2, are primarily local ized to the lung, where they activate a local and systemic inflammatory response and denude the alveolar lining. The resulting hypoxemia and systemic inflammatory response injure 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 conse quences. Repair of the damaged lung and distant organs represents a major challenge to proteostasis that might be overcome in younger individuals but fails 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 Balch WE et al: Adapting proteostasis for disease intervention. Science 319:916, 2008. Balchin D et al: In vivo aspects of protein folding and quality control. Science 353:aac4354, 2016. Chiti F, Dobson CM: Protein misfolding, amyloid formation, and human disease: A summary of progress over the last decade. Annu Rev Biochem 86:27, 2017. Costa-Mattioli M, Walter P: The integrated stress response: From mechanism to disease. Science 368:eaat5314, 2020. Eisele YS et al: Targeting protein aggregation for the treatment of degenerative diseases. Nat Rev Drug Discov 14:759, 2015. Finley D, Prado MA: The proteasome and its network: Engineering for adaptability. Cold Spring Harb Perspect Biol 12:a033985, 2020. Labbadia J, Morimoto RI: The biology of proteostasis in aging and disease. Annu Rev Biochem

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