Protein Quality Control in Neurodegenerative Disease

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The accumulation of misfolded proteins is a common feature of many neurodegenerative diseases. These observations suggest a potential link between these disorders and protein quality control, a collection of cellular pathways that sense damage to proteins and facilitate their turnover. Consistent with this idea, activation of quality control components, such as molecular chaperones, has been shown to be protective in multiple neurodegenerative disease models. In addition, key studies have suggested that quality control deteriorates with age, further supporting a relationship between these processes. In this chapter, we discuss the evidence linking neurodegeneration to quality control and present the emerging models. We also speculate on why proper quality control is so difficult for certain proteins.

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A common feature of many neurodegenerative diseases is the accumulation of misfolded proteins. As discussed in the previous chapters, the predominant offending protein is distinct in each disease (e.g., polyglutamine-expanded huntingtin in Huntington's disease). These disease-associated proteins are prone to misfolding, accumulation, and aggregation. More specifically, oligomers of these misfolded proteins are thought to cause gain-of-function proteotoxicity through a number of mechanisms.

These observations suggest that protein quality control might play a role in neurodegenerative disease.[1–4] Briefly, quality control is a term used to describe a collection of pathways responsible for monitoring protein integrity.[5–7] This system comprises multiple "arms," including the molecular chaperones, the unfolded protein response (UPR) system, the autophagy–lysozome pathway, and the ubiquitin–proteasome system. Together, these components assist in most stages of a protein's life cycle, including its synthesis, folding, assembly/disassembly into complexes, and its degradation and turnover. Through this process, the quality control system continuously monitors the integrity of protein folding, and if misfolding is detected, then triage decisions are made to limit potential proteotoxic damage. In this way, quality control helps maintain proper protein homeostasis (proteostasis).

Based on these observations, an emerging model is that the cellular capacity for quality control is one important variable in determining the onset, severity, and progression of many neurodegenerative diseases. Why do specific proteins chronically misfold in these patients? Why are they not removed or detoxified? Does the capacity of quality control influence neurodegeneration?

I. Overview of Protein Quality Control

To better understand the links between neurodegenerative diseases, protein folding, and quality control, we begin with an overview of the interrelated "arms" of the quality control system. Each of these topics is associated with an expansive and informative literature. We include here key references and recent reviews to provide the foundation for discussing how these systems impact neurodegenerative disease.

A. Molecular Chaperones

The molecular chaperones are a large family of highly conserved proteins that assist in quality control through their roles in protein folding and turnover. One major, ubiquitous class of molecular chaperones is the heat shock proteins,[8,9] so named because the expression of these proteins is sensitive to stress, such as elevated temperature and oxidative damage. However, most heat shock proteins are also expressed under normal conditions and their activities are

critical for normal quality control. This property is highlighted by the fact that some of the abundant heat shock proteins make up 1–2% of the total cellular protein under resting conditions.

The heat shock proteins are named by their approximate molecular weights, with the common classes being Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and the small heat shock proteins. These classes share no apparent sequence or structural homology. Instead, they seem to occupy specific niches in protein quality control. For example, the Hsp100-type chaperones are AAA^+ ATPases that are thought to be primarily involved in protein disaggregation and the primary folding of select substrates.[10,11] Hsp90 has been shown to bind largely folded substrates, such as nuclear hormone receptors and some kinases, to protect them from degradation and assist their remodeling in the presence of ligands.[12] Hsp70 is a family of 70-kDa chaperones that are believed to be involved in primary folding and triage decisions, linking molecular chaperones to the autophagy and ubiquitin–proteasome systems.[13,14] Small heat shock proteins, such as Hsp27 and Hsp33, are ATP-independent chaperones that appear to have specific roles in blocking protein aggregation during stressful conditions.[15] Additionally, these individual classes of heat shock proteins often operate together. For example, Hsp70 and Hsp90 work together to regulate the activity and stability of hormone receptors[12] and they can "hand" substrates to each other.[16] Similarly, Hsp70 and the small heat shock protein Hsp27 bind a model misfolded protein, cystathionine synthase, and their relative availability dictates whether it will be folded or degraded.[17]

The various activities of the chaperones arise from their direct physical contacts with substrates. Consequently, the heat shock proteins are thought to have only loose substrate selectivity, binding rather promiscuously to exposed hydrophobic regions.[18,19] Consistent with this idea, many of the heat shock proteins have hydrophobic "patches" that act as cognate surfaces for binding to aberrantly misfolded proteins. Hsp70 family members contain a hydrophobic pocket capable of binding 8–10 consecutive, nonpolar amino acids in an extended conformation.[20] Other chaperones, such as members of the GroEL or Hsp100 class, assemble into large, homo-oligomeric structures with spacious central cavities. Substrates encapsulated in these cavities are exposed to dynamic, hydrophobic surfaces that facilitate folding or degradation.[11] These chaperones, including the ClpP and ClpX AAA^+ ATPases, have remarkable substrate promiscuity, with only a few peptide-like features required for efficient recognition.[21] Interestingly, other chaperones, such as trigger factor, engage their substrates via polar contact surfaces.[22] Trigger factor also binds its substrates using a relatively large surface, perhaps allowing recognition of many different nonconserved substrates by facilitating multipoint binding, thereby distributing the binding free energy. Consistent with this idea, other chaperones are enriched for intrinsically disordered regions, perhaps providing them with the flexibility to interact with large areas of variable cognate surfaces that possess different topologies.[23]

Anfinsen suggested that protein folding information is coded in the amino acid sequence.[24] In large part, folding proceeds through internalization of hydrophobic residues and formation of other favorable contacts, which make the folded state energetically favorable. This idea is commonly represented by a folding trajectory energy diagram, in which an ensemble of initial elongated structures proceeds through key intermediate states (or "saddle points") to eventually arrive at the low-energy folded structure (Fig. 1). Failure to proceed on this route, either through a thermodynamic or kinetic trap, results in stalled folding and accumulation of an intermediate. Because folding is driven, in part, by internalization of hydrophobic residues, these folding intermediates are often expected to have exposed hydrophobic regions. Thus, they pose a potential danger to the cell because they can be prone to energetically favorable self-association, especially in the crowded cytosol or ER lumen. To combat this possibility, multiple systems exist for assisting in protein folding in cells. For example, a chaperone apparatus facilitates cotranslational folding[25] and the surface topology of the ribosome seems to play a productive role in this process.[26] In addition, other members of the molecular chaperone system have specific roles during conditions of stress, such as thermal or osmotic shock. Environmental stressors have the potential to "reset" the folding landscape and create conditions that melt sensitive proteins. Thus, stress conditions require additional chaperone activities in the cytosol, ER lumen, and other

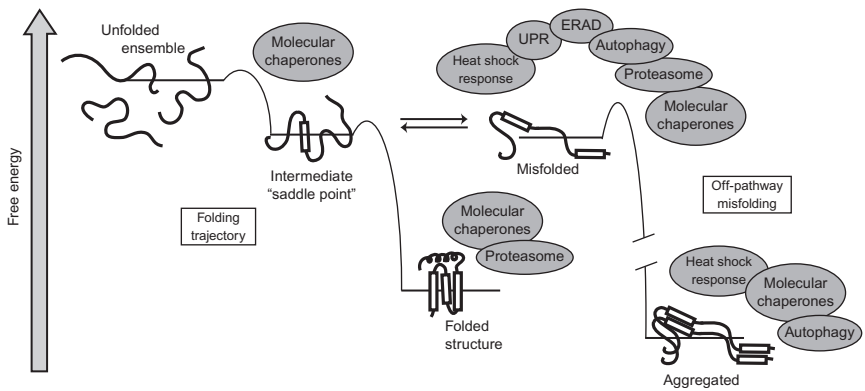

FIG. 1. Oversight of protein folding, misfolding, and turnover by cellular quality control. A simplified schematic of the energetically favorable folding trajectory is shown, starting with an ensemble of unfolded states, proceeding through a folding intermediate (saddle point), and continuing to the low-energy folded state. After folding, turnover is used to recover amino acids. Failure to proceed on the folding trajectory can lead to accumulation of off-pathway misfolded proteins, which can subsequently aggregate. As shown, the various components of the quality control network monitor each aspect of this process.

compartments. A family of stress-inducible chaperones appears to be particularly important in blocking denaturation, inhibiting aggregation, and favoring protein refolding.

In all these activities, molecular chaperones typically bind to hydrophobic regions, shielding exposed hydrophobic patches from bulk solvent and nonproductive interactions. Of course, protein:chaperone contacts also create a natural block to native protein folding because the chaperone-bound hydrophobic regions typically need to be buried in the final folded protein structure. Thus, it is important that protein–chaperone interactions be reversible and, for most chaperones, reversibility is driven by ATP binding and hydrolysis.[9] For example, electron microscopy studies have uncovered the dynamic, nucleotide-dependent cycling of Hsp90 structure.[27] Briefly, apoHsp90 exists in an open dimeric form, capable of loosely binding to substrates. Binding to ATP closes the dimer and increases substrate affinity, while hydrolysis of nucleotide triggers conformational changes that eventually lead to ADP and substrate release. This reversibility is critical because it allows the released substrates another chance to proceed down the folding trajectory. The classic example of this mechanism is the GroEL–GroES chaperone system, which traps misfolded proteins in its interior cavity and uses ATP hydrolysis to drive conformational changes that iteratively expose the substrate to relatively hydrophobic and hydrophilic environments, facilitating translocation, denaturation, and attempts at refolding.[11]

It should be emphasized that, although this discussion is largely focused on initial folding events and chaperone activities associated with stress, chaperones also have important activities on "post-folding" substrates and advanced-folding intermediates. For example, they assist in assembly/disassembly of protein complexes (e.g., clathrin), the transport of polypeptides across membranes (e.g., into the mitochondria), and in the remodeling of active sites during ligand engagement (e.g., nuclear hormone receptors). In addition, many chaperones, especially of the AAA^+ ATPase class, have disaggregation activity, permitting recovery of substrates from off-trajectory fates or storage forms.[10] In this case, ATP turnover can be used to actively "pull apart" aggregated proteins, facilitating their mobilization, recovery, or triage.[11] Thus, chaperone activity is required during many stages of the lifecycle of a protein, whenever hydrophobic regions could be inappropriately exposed.

The folded state of a protein can be partially described by a thermal melting curve, with an associated K_m value which indicates the stability of its protein fold. Each native wild-type protein will be associated with a specific K_m value and a ΔG of folding (Fig. 2). However, errors in translation produce unintended amino acid substitutions on the order of one per 100 amino acids.[164] In addition, this value does not include cumulative errors in mis-aminoacylation or DNA and RNA synthesis. Thus, most (or all) proteins will accumulate errors and a majority of these mutants are likely to adversely impact folding.

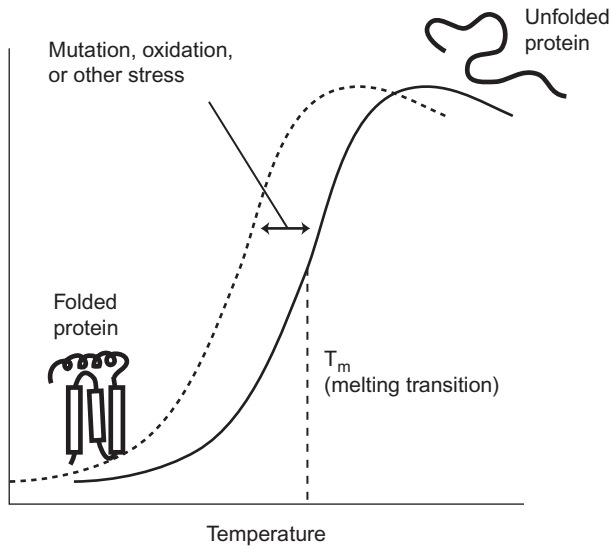

FIG. 2. Protein stability and the effects of mutation and stress. Mis-incorporation of amino acids, natural mutations, environmental stresses, and disease-associated conditions might create scenarios that destabilize proteins and favor their denaturation. As shown in Fig. 1, this process can favor accumulation of misfolded and aggregation-prone intermediates.

Chaperones protect against this problem by facilitating conditions that allow for robust folding across a range of K_m values. This situation could explain why chaperones are so abundant and why there are so few "free" chaperones. Essentially, chaperones are thought to buffer against intrinsic errors in protein synthesis, as well as changes in temperature or environment. Finally, this system creates a clever solution for living organisms because these same "errors" in protein folding are also essential for evolution. Accordingly, chaperones have been found to play an important role in molecular evolution. In a series of illuminating experiments, Rutherford and Lindquist found that mutations in Hsp90 allowed previously silent phenotypic variations to emerge in flies.[28] Interestingly, when these phenotypes were enriched, they eventually became independent of Hsp90, suggesting one possible mechanism by which chaperones produce conditions favorable for evolution of new traits.

B. Heat Shock Response

Although quality control is important for normal proteostasis, cells must also be responsive to acutely stressful situations, such as temperature variations, periods of rapid growth, high demands on secretion of extracellular factors, or oxidative injury. Under these conditions, the total folding and triage capacity of the cell might not match the needs under that particular set of

environmental conditions, necessitating the upregulation of the heat shock proteins and other quality control components. This process is typically called the heat shock response (HSR) and it is controlled, in large part, by the transcription factor, heat shock factor 1 (HSF1). HSF1 is normally bound to Hsp90 in an inactive and monomeric state in resting cells.[29] Accumulation of misfolded proteins, which might be associated with a sudden change in the folding environment, leads to preferential binding to HSP90 and subsequent release of HSF1. HSF1 then forms a trimer and gets localized to the nucleus, where it activates transcription of heat shock proteins and many other pro-survival factors.[30,31] Thus, HSF1 is a critical component of quality control, acting both as a sensor and as a master transcriptional regulator. In addition, its activity appears to help coordinate stress responses in multicellular organisms.[32]

Although heat shock proteins are abundant in resting cells, HSF1 activation significantly elevates their numbers. For example, Hsp90 can comprise 1–2% of total cellular protein under resting conditions and as much as 10–20% after heat shock. These observations suggest that the HSF1-responsive transcriptional programs are highly tuned to deal with stress. In addition to its roles in stress, its activity appears to be developmentally regulated, with the quality control capacity of the cell/organism matched with the required mitotic or metabolic rate. Moreover, this capacity is likely adjusted to respond to specialized functions, such as high secretion rates in active pancreatic beta cells. Thus, the HSR, although important for response to stress, serves many purposes in normal physiology.

HSF1 activity appears to play a critical role in cancer and has emerged as a possible therapeutic target.[33] Many HSF1 targets, such as molecular chaperones and other quality control components, play important roles in apoptosis.[34,35] Typically, these roles are associated with pro-survival responsibilities that support cellular viability. For example, under pathophysiological conditions of unrestricted growth (e.g., cancer), high HSR activity is likely conducive to a strongly pro-folding and pro-survival environment. In turn, these conditions would be expected to support high rates of protein synthesis and net stabilization of oncogene products. However, it is also clear that quality control pathways can promote apoptosis, especially in the apoptosis "arm."[36] Thus, any discussion of the role of quality control in neurodegenerative disease should be tempered by a realization that high folding capacity is not a universally favorable goal. The key is proper balance among protein synthesis, folding, function and turnover.

C. Unfolded Protein Response

While the HSR occurs in answer to the accumulation of misfolded proteins in the cytosol, the UPR serves an analogous function in the ER.[37,38] This process involves at least three distinct signaling pathways, each regulated through activation of a membrane-bound receptor, namely, IRE1, PERK,

and ATF-6, which monitor protein folding in the ER lumen. In resting cells, these receptors are bound to the ER-resident Hsp70 isoform, BiP or Grp78. In a mechanism conceptually similar to that discussed for HSF1, accumulation of misfolded proteins in the ER is thought to release the receptors from BiP, activating them and triggering a series of signaling cascades. The outcome of these pathways is that protein synthesis is slowed and the expression of quality control components, such as BiP and calnexin, is elevated. Thus, this system monitors folding capacity in the ER and adjusts it in response to flux through the organelle. In addition, the UPR increases the levels or activity of proteins needed for removing misfolded substrates by ER-associated degradation (ERAD)[7] and it initiates an apoptosis program if prolonged stress is detected.

Key targets of the UPR, and essential components of protein quality control in the ER lumen, are the protein disulfide isomerases (PDIs). These chaperones play specific roles in protein folding by facilitating the formation of proper disulfide linkages. They lower the barrier to disulfide interchange by forming covalent adducts with substrate proteins and, thus, assist in the search for the correct combination.[39,40] This step can often be rate-limiting for protein folding, creating a special requirement for this activity along the folding trajectory. Similarly, a family of peptidyl prolyl isomerases (PPIases) acts on another common "saddle point" event in protein folding, namely, isomerization of proline residues.[41] These activities are essential components of the UPR pathway, as they greatly speed the folding process and reduce the dwell time for potentially proteotoxic folding intermediates.

The lumen of the ER provides a number of specific challenges to protein folding, including its high protein content, strict requirements for cargo sorting, and the need for proteins to be first unfolded before they enter the space. Similar challenges are imposed in other organelles. However, less is known about quality control in some compartments. Extensive work has been performed on mitochondrial quality control, which provides an illustration of how folding and triage may be handled.[42,43] The major components of mitochondrial quality control include (a) proteases of the AAA^+ ATPase family (such as Lon protease), which degrade damaged and misfolded proteins; (b) ubiquitination and reverse transport of substrates to the cytosolic proteasome system; (c) organelle-specific chaperones; and (d) mitophagy or degradation of entire mitochondria. In addition, reactive oxygen species (ROS) pose a constant danger to the proteins, lipids, and nucleic acids of the mitochondrium, requiring a number of quality control components that focus on antioxidative activities (such as methionine sulfoxide reductases).[44] For example, the Lon protease seems to specifically recognize and degrade aconitase that has been oxidatively damaged.[45] Even with these measures, damaged proteins tend to accumulate in the aged mitochondria of some tissues, perhaps because of aging-related decreases in Lon activity.[46]

D. Autophagy–Lysosome

If a protein is targeted for triage by the quality control systems, cellular degradation pathways are used to recycle amino acids and avoid accumulation of potentially toxic aggregates. These pathways include macroautophagy, a process of enclosing cytoplasmic cargo in a double-membrane vesicle (the autophagosome) and delivering the contents to the lysosome.[47–49] Autophagy is commonly induced under nutrient-starvation conditions, allowing the cell to recover amino acids and other metabolites. It is initiated by phosphoinositide 3-kinase activity and an initiating factor, beclin (Atg6 in yeast). Through the activity of the ubiquitin-like protein, LC3, and other autophagy-related proteins, the autophagosome membrane assembles and is fused with the lysosome. This system can degrade entire organelles (e.g., damaged mitochondria) and substantial volumes of cytosol, including protein aggregates that are resistant to other degradation pathways. The other major autophagic pathway is called chaperone-mediated autophagy (CMA). This process presumably involves more discrete selection of misfolded substrates by chaperones. Using signals that are beginning to be understood, Hsp70 family members seem to recruit misfolded or damaged proteins to LAMP2 in the lysosomal membrane and facilitate their degradation. Recent reviews provide additional molecular details into these two autophagy pathways.[50–52]

Regardless of the entry pathway, cargo proteins are delivered to the lysosome for degradation. The lysosome is a membrane-bound organelle that sequesters a number of hydrolase activities (especially proteases, lipases, amylases, and nucleases) and allows local generation of a highly acidic compartment (∼pH 4.5). Of particular relevance to neurodegenerative disease, proteotoxic aggregates are delivered to the lysosome by autophagy pathways.[53,54] This might be one of the few ways to remove large, preformed aggregates from the cell. Moreover, lysosome dysfunction is often critical in neurodegenerative disease etiology, suggesting that this compartment might be either overutilized or otherwise compromised.

As mentioned above, there must be a balance between pro-survival activities and unfettered cell division and growth. As concrete evidence of this idea, flux through the autophagy pathway is often elevated in cancer cells,[55] while failure to correctly induce autophagy appears to be linked with Huntington's disease.[56] This balance appears to be critical in disease. For example, autophagy in neurons initially appears to play a pro-survival role, while aging converts this process to a pro-death role.[57] Similarly, the increased autophagic flux in cancer cells allows rapid turnover and recycling in a system that is likely to produce more "mutant" proteins, but activation of autophagy is also pro-apoptotic beyond an ill-defined threshold. This balance appears to involve communication with other protein quality control pathways, such as the UPR.[57]

E. Ubiquitin–Proteasome

The proteasome is a large, cytoplasmic, cylindrical complex containing three distinct proteolytic activities. The most common form of the proteasome is the 26S particle, which is composed of a 20S core particle and two 19S "cap" particles. The 20S subunit contains a channel with chymotrypsin-like, trypsin-like, and peptidyl–glutamyl hydrolyzing activities. Together, these activities degrade substrate proteins that enter the 20S cavity. The 19S particles interact with the two ends of the 20S particle, acting as gatekeepers by specifically recognizing ubiquitin-modified substrates and other proteins tagged for destruction. In addition, these 19S particles also have ATP-dependent unfolding activity. This activity is important because proteins must be partially denatured to gain access to the relatively narrow (≈50 Å) channel of the 20S particle. It has been hypothesized that larger aggregates, such as those commonly observed in neurodegenerative disease, might be resistant to the proteasome activity, in part because of their relative resistance to unfolding.[54] Although the proteasome is primarily located in the cytosol, potential substrates are also trafficked to this system from other compartments, including the ER, mitochondria, and plasma membrane.[58–60]

Substrates are tagged for degradation by either polyubiquitination or through ubiquitin-independent mechanisms.[61] It is not yet entirely clear how all substrates are selected for degradation by these pathways. However the N-end rule pathway provides some clues. The N-end rule describes an observation that the identity of the extreme N-terminal residue of a protein can dictate its relative stability in the cytosol.[62] In this pathway, proteins with the appropriate residues, such as Phe, Trp, and Leu, at their N-termini are relatively rapidly turned over via ubiquitin-dependent proteasomal clearance. However, other mechanisms also seem to contribute to determining stability, and a unifying theorem of turnover has not yet emerged. For example, ubiquitin-dependent mechanisms appear to also operate in autophagosome/lysosomal targeting. Further, the exact roles of the molecular chaperones in choosing substrates for degradation are not yet known. These questions will likely be of critical importance for understanding how quality control impacts neurodegenerative disease.

F. Summary of Protein Quality Control

Together, the protein quality control components (e.g., chaperones, UPR, autophagy, ERAD, proteasome) provide critical oversight by facilitating folding, reducing accumulation of proteotoxic substrates, and helping to maintain proteostasis (see Fig. 1). In neurodegenerative disease, misfolded substrates accumulate, suggesting a deficit in the capacity of quality control or some

failure to properly triage toxic substrates. These observations raise intriguing questions about the roles of quality control pathways in neurodegeneration, proteostasis, longevity, and aging. How does chronic dysfunction of quality control impact disease progression? How do changes in one "arm" of quality control impact the entire system? Which components of quality control might serve as the best therapeutic targets for neurodegenerative diseases? Can these targets have influence over multiple types of neurodegenerative disease? In the next sections, we examine representative proteotoxic proteins whose accumulation is associated with specific neurodegenerative diseases. The intent is to provide a brief overview, while recent reviews (*vide infra*) can be consulted for more in-depth analysis.

II. Proteotoxic Proteins and Quality Control

A number of proteins are associated with specific neurodegenerative diseases. These proteins share common traits, such as proteotoxicity and the propensity to form aggregates *in vitro* and *in vivo*. Many of these proteins form amyloids, a specific type of protein aggregate that has features of high β-sheet content and reactivity with histological dyes, such as thioflavin T.[63] A few of these substrates are relatively newly characterized (e.g., TDP-43) and less is known about their relationships with protein quality control. Here, we focus on some of best characterized proteotoxic systems in which clear roles for protein quality control have been elucidated. Through these examples, we will outline some general themes that might be important for future consideration.

A. Polyglutamines

Some proteins, including huntingtin, androgen receptor, and ataxin-2, contain stretches of contiguous glutamine residues.[64] These polyglutamine regions are typically less than 35 residues in length. However, expansion of these regions to 40 or more residues makes them prone to aggregation.[65] Interestingly, polyQ-expanded proteins have been found to be relatively resistant to proteolysis and they are poor substrates for the proteasome, suggesting that they might avoid proper turnover,[54] although this conclusion is controversial.[66] In key studies, the Morimoto group has shown that accumulation of polyQ aggregates can affect cellular proteostasis, allowing misfolding of proteins that, in the absence of the polyQ protein, would be folded and stable.[67,68] Some of these aberrant interaction partners seem to include important transcription factors and chaperones.[69] In fact, a network of polyQ-interacting proteins appears to be required for toxicity in a yeast model.[70] Together,

these studies suggest that aggregation of polyQ, its avoidance of proper degradation, and its ability to disrupt global proteostasis might contribute to disease pathogenesis.

In support of this idea, numerous genetic and biochemical studies have suggested that increasing the capacity of the protein quality control pathways can block polyQ-related pathology. For example, in yeast, worm, and fly models, polyQ-associated phenotypes are suppressed by concurrent overexpression of molecular chaperones, including Hsp70 and Hsp40.[71–77] The interactions between the polyQ and chaperones appear to be dependent on molecular features of the polyQ. Early studies established that the interactions with Hsp70 and Hsp40 were dependent on polyQ length.[78] Moreover, Hsp27 was found to specifically bind to the regions flanking the polyQ expansion to impact self-assembly and toxicity.[79,80] In one recent and interesting example, the Hsp70–Hsp40 complex was found to selectively associate with small aggregates of huntingtin polyQ, suggesting roles of these chaperones during the critical oligomeric stages of aggregation.[81] Consistent with these general ideas, knockdown of Hsp70 exacerbates polyQ phenotypes in flies[82] and mice.[83] In addition to the direct effects of chaperones on the ability of polyQ proteins to aggregate, these factors also can facilitate their degradation. For example, some Hsp40s, such as HSJ1a, increase ubiquitylation of polyQ in neuron models.[84] Together, these studies suggest that the levels of chaperones and the overall cellular folding capacity might influence the propensity for polyQ aggregation and its cellular impact. However, in most cases, the exact molecular mechanisms of protection are not yet clear. How do chaperones guide polyQ substrates to degradation pathways? What are the exact molecular features being recognized? Why does this system fail in diseased patients?

In addition to molecular chaperones, other components of the quality control pathways appear to impact the assembly and toxicity of polyQ fragments. For example, large polyQ aggregates are effectively cleared from the cytosol (but not in the nucleus) by autophagy.[85] Further, induction of an HSR, through either genetic or pharmacologic intervention, also mitigates polyQ assembly and phenotypes.[86–90] Bien and Bonini conducted genome-wide screens in flies to reveal both chaperone and ubiquitin pathways as major modifiers of toxicity of polyQ-expanded ataxin-3,[71] and in support of this idea, Zoghbi *et al*. performed yeast two-hybrid studies to implicate these pathways and others in ataxia.[91] Together, these studies have strongly suggested a link between polyQ-associated pathology and the capacity of the quality control network.

B. Microtubule-Binding Protein Tau

Accumulation of hyperphosphorylated tau is associated with a number of neurodegenerative disorders, including Alzheimer's disease (AD), Pick's disease, and frontotemporal dementia with Parkinsonism linked to chromosome

17 (FTDP17).[92,93] Moreover, tau has recently been implicated in mediating the proteotoxic effects of amyloid β-protein (Aβ) in models of AD.[94,95] Thus, there is increasing interest in understanding how tau is processed by cellular quality control systems.

As discussed in Chapter 8, tau is abundantly expressed in the brain, where it is composed of 11 exons that are alternatively spliced into at least six major isoforms. In addition, tau is extensively altered by posttranslational modifications, including phosphorylation and proteolytic processing.[96] Together, these modifications create a structurally diverse population of tau proteins in neurons, but the individual cellular activities and relevance of these isoforms are not yet clear. One common theme, however, is that the hyperphosphorylated and proteolyzed versions accumulate in AD and other neurodegenerative diseases.[97] These forms of tau have a tendency to aggregate, forming insoluble inclusions, termed neurofibrillary tangles (NFTs), and soluble oligomers that have been linked to gain-of-function proteotoxicity in disease.[98] Some of these posttranslational modifications appear to reduce tau's affinity for microtubules, which might further contribute to their tendency to self-associate.

Tau is an intrinsically disordered protein (IDP) and thus it presents many potential opportunities for interactions with chaperones.[23,99,100] Consistent with this idea, the stability and activity of tau appear to be regulated by both Hsp90 and Hsp70. Hsp70 is thought to bind directly to tau and the binding sites have been mapped *in vitro*.[101] Chemical inhibition of either of these chaperones leads to tau's rapid degradation by the proteosome.[102–104] Interestingly, it was recently reported that inhibition of Hsp70 in transgenic mice reduces soluble tau levels and improves cognition, suggesting that this chaperone plays an important role in both controlling tau stability and determining proteotoxicity.[105] However, neither Hsp70 nor Hsp90 works alone in this tau triage process, and many of their associated co-chaperones may also be involved.[106,107] Hsp27 also binds tau and regulates its stability.[108] Proteasomal degradation of tau appears to depend on the E3 ligase CHIP, which forms a complex with both Hsp70 and Hsp90.[102,109] The co-chaperones BAG-1, BAG-2, FKBP52, and PP5 also have been implicated in helping chaperones identify hyperphosphorylated tau.[110–113] PP5 is a particularly intriguing chaperone partner, as it is a protein phosphatase that has been shown to directly act on hyperphosphorylated tau in an Hsp90-directed fashion. In addition to chaperone-mediated clearance by the proteosome, tau also appears to be removed by macroautophagy and CMA, suggesting that multiple cellular pathways can act on this substrate.[114,115] Together, these observations suggest a model in which the cellular levels of tau and its isoforms are tightly regulated by a network of quality control systems. In this model, aging-related decreases in quality control capacity or mutational damage to tau might tip the balance toward accumulation of aggregation-prone and proteotoxic structures.

C. Amyloid β-Protein

Aβ is produced from the proteolytic cleavage of the amyloid precursor protein (APP), and as discussed in chapter Alzheimer's Disease and the Amyloid β-Protein, its deposition into fibrils is characteristic of AD. Like other neurodegenerative disease-related proteins, Aβ self-assembles into aggregates. *In vitro* and in cells, addition of molecular chaperones, including cytoplasmic and ER-resident Hsp70s, Hsp90, various Hsp40s, and small heat shock proteins, can block Aβ aggregation.[116] In worm models, Hsp70 and Hsp16.2 mitigate Aβ-related defects, further supporting a role for quality control.[117,118] Interestingly, the Good group found that multiple small heat shock proteins could block Aβ aggregation, but only Hsp20 was effective at suppressing toxicity.[119] In one recent report, treatment with heat shock appeared to suppress Aβ toxicity, suggesting that multiple quality control pathways might be involved.[120] However, most chaperones and quality control pathways operate inside the cell, whereas Aβ deposition is typically observed in extracellular regions of the brain. Thus, it is not yet clear how chaperones might impact AD. One possibility is that they impact APP processing in the secretory pathway[121] or that induction of a stress response provides general protection against apoptosis.[122]

D. α-Synuclein

As discussed in chapter Molecular Insights into Parkinson's Disease, Parkinson's disease (PD) is a complex set of disorders that involve defects in energy metabolism and are associated with aggregation of α-synuclein into Lewy bodies. Molecular chaperones, including Hsp70, Hsp27, and crystallin, have been found to be associated with α-synuclein in Lewy bodies.[123–125] Further, quantitative proteomics have revealed a number of other quality control components in these aggregates.[126] Finally, mutant forms of α-synuclein have shown impaired susceptibility to degradation by autophagy, suggesting that they might evade normal turnover.[127]

Similar to what has been observed with polyQ substrates, Hsp70 and Hsp90 seem to interact with α-synuclein during relatively early stages of self-assembly.[128,129] Moreover, overexpression of Hsp70 in mouse models also suppresses α-synuclein aggregation and toxicity.[130] The interaction between α-synuclein and Hsp70 occurs through the hydrophobic domain of α-synuclein, consistent with other chaperone substrates.[131] However, recent evidence from a mouse model of α-synucleinopathy suggests that overexpression of Hsp70 is not protective.[124,132] Even though the direct link to Hsp70 is found to be insufficient, other quality control and metabolic pathways appear to impact α-synuclein toxicity, as elucidated by genetic screens[133] and heat shock studies in a yeast model.[134] In addition, the small heat shock protein, Hsp27, seems to be a particularly effective anti-α-synuclein agent[135] and pharmacological activation

of a stress response in flies and mice suppresses α-synuclein-associated pathologic effects.[136–138] Similar to what has been observed in other disease models, it seems likely that the effects of protein quality control on α-synuclein toxicity will involve a combination of direct effects on folding, turnover, and subcellular trafficking. Which of these pathways are most important in the pathogenesis of the disease is an important question for future studies.

E. Prions

Prions are a family of proteins found in mammals (chapter The Complex Molecular Biology of Amyotrophic Lateral Sclerosis (ALS)), yeast (chapter Tau and Tauopathies), and prokaryotes that exhibit at least two structural forms, one of which is able to propagate conversion of the other.[139] The conversion of prions to the infectious state proceeds with significant refolding and conformational changes in the prion fold, suggesting that chaperones and components of the quality control system may be associated with this process. Consistent with this idea, extensive work on prions has been done in yeast systems and a clear role for multiple molecular chaperones and quality control systems has emerged.[140] Briefly, *Saccharomyces cerevisiae* harbor a number of natural prions, including Sup35 and Pin1. Studies on these prions have converged on a model in which Hsp70, Hsp40, and Hsp26 are required for the initial "loosening" of prion fibrils, followed by the action of Hsp104 to fully disassemble them into shorter fragments.[141–143] This process appears to be required for propagation of the prion, as knockouts of Hsp104 and Hsp70s, including Ssa and Ssb family members, have altered transmission to daughter cells.[144] However, the relationships between chaperones and prion processing are complex and still being elucidated. For example, chaperones actively avoid formation of toxic intermediates during prion assembly and disassembly.[144,145] Certain Hsp40 proteins can detoxify Rnq1 by prompting formation of aggregates and limiting accumulation of toxic prefibrillar structures,[145] a result that is consistent with experiments performed in polyglutamine disease models.[80] At the same time, supraphysiological overexpression of Hsp70 and small heat shock proteins can suppress prion propagation,[146] presumably through enhanced degradation and altered balance between fibril assembly and disassembly.

Compared to the work performed in yeast, fewer studies have explored quality control in human or mammalian prion systems. Prions cause a number of neurodegenerative diseases in humans, including Creutzfeldt–Jakob disease (CJD). One interesting study has shown that mouse knockouts of HSF1 are more susceptible to toxicity by prions, suggesting broad roles for the HSR.[147] Because prions operate through a folding transition and their infectivity is dependent on a distinct folding state, it seems likely that chaperones will be found to have additional important activities in determining susceptibility to infection. For example, the chaperones GroEL and Hsp104 were found to

promote conversion of a mammalian prion to its protease-resistant infectious state when the reactions were seeded with some of the disease-associated protein.[148] In addition, Hsc70 bind to prion protein (PrP), suggesting a potential role for this chaperone.[149] However, it should be noted that the effects of protein quality control components on directly balancing the folding, conformational transitions, turnover, and propagation of prions are likely supplemented by more global effects on antiapoptotic signaling pathways.

III. Reflections and Prospectus

From these observations and many others, it is clear that protein quality control plays a critical role in determining the fate of substrates whose accumulation is associated with neurodegeneration. Not surprisingly, these realizations have led to a second wave of interesting and provocative questions and, in many ways, the studies outlined in this chapter are only the beginning.

(1) Why are older individuals more prone to neurodegenerative diseases? Do protein quality control pathways fail over time? Accumulating evidence indicates that key proteostasis network pathways, including those involved in protein quality control, are compromised in older individuals. For example, the HSR is eroded as a function of aging,[6,88,150,151] suggesting that some arms of quality control become inoperative or less effective. Why might this be? Perhaps, epigenetic changes could lead to impaired HSR or UPR. For example, HSF1-binding sites in DNA appear to change with age.[152] In addition, accumulation of oxidative damage might overload quality control systems, making it easier for disease-related substrates to avoid detection. In this scenario, increased loads of proteotoxic proteins might increase the demand for quality control, which would be expected to increase the basal stress response and strain the ability to induce robust HSR. In turn, these chronic imbalances might decrease the efficiency of autophagy, induce lysosomal dysfunction, and reduce proteasome activity by shifting quality control responsibilities to these "arms" of the system. Despite this speculation, very little is known about the molecular mechanisms that link neurodegeneration to aging, and, similarly, our knowledge of the relationships between aging and protein quality control is still incomplete.

(2) How are certain disease-related substrates (e.g., tau, polyQ) able to avoid proper quality control? In some cases, the answer might be related to specific mutations that alter the molecular recognition between chaperones and the substrates. For example, the 42-amino acid version of Aβ is more prone to aggregation than the 40-amino acid form[153] and, concurrently, mutations that alter the ratio of Aβ42 to Aβ40 can impact disease (as

discussed in the chapter entitled Cerebral Amyloid Angiopathy). Another interesting case involves disease-associated mutations in the ER. Certain alleles of glucocerebrosidase (GCase) reduce the levels of functional enzyme by triggering premature degradation.[154] Interestingly, some of these GCase mutants do not appear to activate the UPR despite the fact that they are relatively defective for folding. Together, these observations suggest that GCase mutants may partially avoid normal quality control decisions, which leads to Gaucher disease.

In some cases, resistance of proteotoxic aggregates to normal clearance mechanisms might eventually lead to their accumulation. For example, some polyglutamine aggregates are thought to be relatively resistant to proteasomal degradation.[54] This scenario might lead to irreversible deposition, allowing the proteotoxic protein to accumulate unchecked. In turn, deposition of these aggregates has been shown to disrupt more global proteostasis by recruiting bystander proteins, especially intrinsically unfolded or metastable proteins.[155] Therefore, allowing substrates to evade degradation can have catastrophic consequences beyond just the intrinsic proteotoxicity of the aggregate.

We understand very little about how substrates are chosen for degradation, even under normal conditions. Thus, our ability to understand why some substrates accumulate in neurodegenerative disease is also limited. Perhaps these disease-related substrates share some unrecognized motif (e.g., high β-sheet content?) that is particularly troublesome for the quality control machinery. Perhaps the disease-related substrates are efficient at "hiding" their hydrophobic motifs, allowing them to appear normal and evade turnover. Do some proteins aggregate fast enough to evade quality control? For example, specific mutants of tau are especially prone to rapid aggregation and they are associated with FTDP17.[97] These types of questions will likely be of critical importance in the future of neurodegenerative disease research.

(3) Why are neurons sensitive to accumulation of misfolded proteins? Do different cells or tissues have different capacities for quality control? Do different cells have different proteotoxic protein loads? Do tissue-specific aging patterns alter the folding capacity of the brain? It seems likely that different tissues have distinct quality control capacities. For example, Hageman and Kampinga have demonstrated tissue- and organelle-specific differences in the expression levels of some molecular chaperones,[14,156] perhaps creating local differences in folding environments. In addition, postmitotic cells might be prone to accumulate some types of DNA damage as they age,[157–159] which could strain protein quality control systems. Still, these questions are under active study and it seems likely that multiple mechanisms will emerge.

(4) How do deficits in protein quality control contribute to neurodegenerative disease? It is thought that one central issue here is a balance between quality control and the proteotoxic load. In healthy individuals, balance appears to be maintained, with the capacity of the quality control systems capable of dealing with changes in protein loads. However, imbalance might be triggered by increases in the amount of misfolded protein combined with decreases in the capacity (e.g., perhaps aging-related?) of quality control systems. Interestingly, one would expect that the loss of balance would be different in different people (or tissues or cells) because they each have a different proteotoxic load, different accumulated amounts of DNA damage, and different levels of quality control activity. For example, some individuals may have a relatively "weak" oxidative stress response. These persons might be more susceptible to developing sporadic PD if they also carry other risk alleles or were exposed to high levels of environmental toxins. In this case, the capacity of quality control might be considered permissive for PD, but the same person with a "strong" oxidative stress response, all other things being equal, might not have developed disease.

Clearly, when pondering this question, it is critical to appreciate how the quality control components are linked within the proteostasis network.[32] For example, autophagy and proteasome pathways appear to be coregulated, allowing alternative shuttling of substrates into one or the other system.[160,161] Thus, a more useful way to view quality control with respect to neurodegenerative diseases is that pathology likely arises from global failures, not just problems in one "arm" (e.g., autophagy). In other words, a slow decline in total quality control capacity (defined as the additive effects of chaperones, HSR, UPR, autophagy, etc.) might lead to incremental accumulation of misfolded proteins. In turn, these initiating problems might nucleate the misfolding of disease-related targets and bystander proteins. In this scenario, proteostasis eventually collapses, causing rampant aggregation and, in theory, disease.

Another interesting way to view this question is that the quality control systems are not deteriorating *per se*, but that they might be attempting to "rescue" misfolded proteins because they are "misreading" the folding information. This well-intentioned attempt at rescuing a misfolded substrate might occur to the detriment of the health of the organism. In this case, mutations in the disease-related protein might evade even an intact system, potentially having relevance for early onset disease. Regardless of the mechanisms, it is likely that each of these possibilities occurs under some conditions and greater emphasis on integrated systems biology approaches might accelerate discovery in this area.

(5) Can protein quality control be a therapeutic target for neurodegenerative disease? One area of active investigation is the search for compounds that boost overall folding capacity through stimulating HSR or UPR pathways. For example, promising early stage efforts are focusing on activating HSF1.[4,162] Thus, one goal of these efforts is to counterbalance aging- or oxidation-related decreases in folding capacity. Other efforts seek to control folding capacity by more specifically regulating the environment in specific organelles. For example, drugs directed to ER calcium channels can be used to increase calcium levels in the lumen, altering folding in this organelle and enhancing the levels of glycocerebrosidase mutants.[163] These studies suggest that a wide variety of potential drug targets exist in the broad realm of quality control pathways. The challenge will be how to put these networks together to predict off-target effects and identify the best (e.g., least toxic and most effective) targets for neurodegenerative disease.

One of the intellectually fascinating aspects of neurodegenerative diseases is that these disorders appear to be uniformly associated with some aspects of protein misfolding and accumulation. Thus, even though examples such as Alzheimer's and Huntington's diseases are clinically distinct and they manifest in different symptoms, they share some fundamental protein biochemistry. For example, fibrils formed by tau, Aβ, and all other amyloid proteins share a common core structure. These commonalities are exciting because they suggest related molecular mechanisms of formation. Further, as mentioned above, quality control needs to be somewhat nonselective in order to monitor the folding of an entire proteome. Can this same lack of selectivity be leveraged to impact AD, HD, PD, and other neurodegenerative disorders using a shared strategy? Clearly, more work is needed to answer these questions, but the basic biology and preliminary translational studies have provided tantalizing clues. For example, some evidence suggests that chemical targeting of chaperones can reduce protein aggregation in mouse models of both HD and AD.[88,105]

The theme in all of these early attempts at quality control-based drug discovery is to artificially boost or manipulate quality control. However, it is not yet clear whether specific substrates can be targeted for increased scrutiny. How do you get the chaperone system to reduce polyglutamine aggregation without impacting global proteostasis? What are the key drug targets to achieve selectivity? Addition insights and the answers to these questions are likely to emerge from ongoing studies into the basic biology of quality control and its relationships with aging and neurodegenerative disease.

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