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Interplay Between Protein Homeostasis Networks in Protein Aggregation and Proteotoxicity

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Abstract

The misfolding and aggregation of disease proteins is characteristic of numerous neurodegenerative diseases. Particular neuronal populations are more vulnerable to proteotoxicity while others are more apt to tolerate the misfolding and aggregation of disease proteins. Thus, the cellular environment must play a significant role in determining whether disease proteins are converted into toxic or benign forms. The endomembrane network of eukaryotes divides the cell into different subcellular compartments that possess distinct sets of molecular chaperones and protein interaction networks. Chaperones act as agonists and antagonists of disease protein aggregation to prevent the accumulation of toxic intermediates in the aggregation pathway. Interacting partners can also modulate the conformation and localization of disease proteins and thereby influence proteotoxicity. Thus, interplay between these protein homeostasis network components can modulate the self-association of disease proteins and determine whether they elicit a toxic or benign outcome.

Keywords

molecular chaperones; protein aggregation; protein misfolding

INTRODUCTION

Numerous conformational diseases are characterized by the misfolding of damaged or mutant proteins that accumulate as intra- and extracellular protein aggregates. The amino acid sequence of the disease protein dictates the particular disease state as well as the effected brain region. Interestingly, the aggregates observed in numerous neurodegenerative diseases including Alzheimer's, the poly-glutamine expansion diseases and transmissible enchapholopathies, share a similar amyloid-like conformation despite divergent primary amino acid sequences. Disease proteins can adopt a non-native, β-sheet-rich conformation as a result of age or environmental stress[1] which enables the exposed β-sheets to stack perpendicular to the elongating fiber axis.[2] Once a critical number of mis-folded protein monomers have associated into a stable structure, the resulting amyloid seed can drive the conversion of native protein into the growing amyloid fibril. Amyloid is defined by an established set of biochemical criteria which include recognition by indicator dyes such as Congo Red or Thioflavin and resistance to protease digestion and detergent solubilization.[3]

Amyloid was widely believed to represent the toxic agent for decades due to its overwhelming presence in the postmortem brains of diseased individuals.[4,5] There are indeed instances where amyloid aggregates are cytotoxic.[6,7] However, recent evidence has challenged the notion that

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amyloid is the primary toxic agent due to the poor correlation between amyloid accumulation and disease progression.[8,9] Furthermore, a number of nonpathogenic proteins such as yeast prions have been shown to form amyloid-like aggregates that are not toxic.[10,11] Thus, intermediates in the aggregation pathway may more appropriately represent the toxic species (see Figure 1).[8,9] Yet the identity of the toxic intermediate structures remains poorly defined. Additionally, the mode of action by which these oligomeric intermediates induce proteotoxicity remains unclear and may include ubiquitin-proteasome system dysfunction,[12] transcriptional deregulation,[13–15] membrane damage,[16–18] and/or aberrant signaling.[19]

Neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U), are characterized by the accumulation of misfolded disease proteins in nonamyloid, ubiquitinated inclusions.[20] Thus, proteotoxicity cannot be due to amyloid formation in these cases. Indeed, a growing body of literature supports the notion that assembly of misfolded disease proteins into tight amyloid-like aggregates may provide a protective mechanism for the cell by preventing the accumulation of toxic misfolded species.[21–23] However, there is limited experimental evidence to suggest that the formation of nonamyloid protein aggregates is protective.[6] Even though the aggregation endpoint differs between the amyloid and nonamyloid conformational diseases, potential intermediate structures and off-pathway byproducts of both aggregation pathways could underlie proteotoxicity and transcend the different disease states.

Many of the disease-causing proteins described herein are ubiquitously expressed throughout the brain yet specific neuronal populations appear more vulnerable to cell death. Thus, proteotoxicity is not merely the result of protein mis-folding and aggregation. Numerous cellular factors embodied in protein homeostasis, intervene to modulate protein mis-folding events.[1] The cellular environment that harbors the different disease proteins plays a crucial role in disease protein stability. In fact, disruptions in the subcellular location of disease-causing proteins are often a critical marker for pathogenesis.[24,25] It is within the different subcellular environments that the disease protein is subject to a multitude of unique protein–protein interactions. In this crowded cellular milieu, transient interactions between proteins with similar sequence or structural elements can significantly alter the conformation and/or location of disease proteins. Similarly, molecular chaperones act within the crowded cellular environment to buffer protein misfolding events. Distinct chaperone networks exist in different subcellular compartments to most appropriately maintain proper protein homeostasis within that organelle.[26] Thus, fluctuation in chaperone availability between different compartments can strongly influence the conformation of the disease protein.[27] The role that individual branches of chaperone networks play in disease progression, is complex and warrants extensive studies on individual disease substrates. Yet the convergence of all the protein homeostasis branches appears to influence the global regulation of disease protein misfolding. Herein, we will begin to scratch the surface of this dynamic interactive network and examine how the interplay between molecular chaperones, protein interaction networks, and subcellular environment ultimately dictate whether a misfolded disease protein is benign or toxic.

ROLES FOR MOLECULAR CHAPERONES IN PROTEIN CONFORMATIONAL DISEASES

Molecular chaperones are part of integrated feedback mechanism which functions to both maintain and restore the global folding state of proteins in the cell during environmental or intrinsic stresses.[27] Molecular chaperones were first identified in *Drosophila melanogaster* due to their increased expression upon exposure of cells to nonpermissive heat treatments and were therefore termed heat shock proteins (HSP).[28] In the ensuing decades, molecular chaperones have been identified in multiple subcellular compartments and include six major subclasses: HSP100, HSP90, HSP70, HSP60, HSP40, and the small HSPs. The diversity of

different chaperone classes and their dynamic regulation by an even more diverse set of cochaperones and cofactors enables a highly specialized network. These chaperone networks act as first line of defense against protein conformational diseases.[29] Conventionally, chaperones suppress the accumulation of misfolded disease protein by promoting refolding or degradation pathways.[30] Yet recent evidence suggests that chaperones can also drive the assembly of misfolded disease proteins into tightly ordered aggregates and thereby reduce the accumulation of soluble aggregation intermediates implicated in proteotoxicity.[21,31,32] Thus, molecular chaperones play multiple roles in the management of protein misfolding and proteotoxicity.

Chaperones Antagonize Aggregation of Disease Proteins

Molecular chaperones can act early in the aggregation process to maintain the solubility of disease proteins. Chaperones can bind misfolded disease proteins and prevent their self-association into ordered, amyloid-like aggregates.[29,33] This concept is exemplified by the well characterized HSP70:HSP40 system that utilizes an ATP hydrolytic cycle for nonnative substrate binding and release. The HSP70 and HSP40 chaperone families are abundantly expressed throughout the cell with 11 and 41 respective isoforms in humans.[34,35] HSP40 proteins are also referred to J-proteins due to the presence of a highly conserved J-domain. Yet a high divergence in the other segments of the HSP40 proteins provides a structurally and functionally diverse class of J-domain containing proteins and enables highly specific substrate recognition properties by the different HSP40 chaperones.[36]

HSP70 cooperates with different HSP40 cochaperone partners to recognize and refold misfolded proteins. Both HSP40 and HSP70 have a high propensity to bind hydrophobic amino acid sequences that might normally be buried in native substrates but become solvent-exposed in the nonnative state.[37] In the absence of chaperones, the exposed hydrophobic stretches of nonnative proteins can result in their self-association into an ensemble of aggregated structures (see Figure 1). HSP40 intervenes by directly binding the misfolded protein and delivering the nonnative substrate to HSP70.[38] Once in complex with HSP70, the J-domain of HSP40 stimulates ATP hydrolysis on HSP70, which increases the binding affinity of HSP70 for the nonnative substrate.[39–41] The nonnative substrate in complex with HSP70 can then be refolded into its native conformation or marked for degradation. Through this hydrolytic cycle, HSP70 and HSP40 can suppress the toxic accumulation of misfolded disease proteins.

Maintaining the solubility of misfolded disease proteins by HSP70 and HSP40 has consistently been shown to suppress proteotoxicity associated with numerous conformational diseases. In particular, extensive work has examined chaperone intervention on the glutamine-encoding expansion diseases,[29] of which there are at least nine different disorders identified to date.[42,43] Utilizing different eukaryotic models, elevated expression of different HSP70 and HSP40 chaperones inhibits poly-glutamine aggregate formation and suppresses proteotoxicity.[44–48] Removal of a particular HSP40 chaperone, Ydj1, converts the normally benign glutamine/asparagine-rich fragment of the Rnq1 prion protein into a less soluble, proteotoxic conformer.[49] Elevated pools of HSP70 and HSP40 also reduced aggregation of the mutant superoxide dismutase 1 protein (SOD1), a causative agent in familial amytrophic lateral sclerosis (ALS), and suppressed neurotoxicity.[50,51] Similar results were obtained in a Parkinson's disease model in which overexpression of HSP70 reduced aggregation of the disease-causing protein, α-synuclein, and suppressed proteotoxicity.[52] Thus, HSP70 and HSP40 maintain the solubility of numerous disease substrates and prevent proteotoxicity in a variety of model systems.

Molecular chaperones can also facilitate the disassembly of protein aggregates into monomers, which can subsequently be refolded or degraded by the cell. The AAA ATPase class of chaperones which include HSP104 in *Saccharomyces cerevisiae* yeast and its homolog ClpB in *Escherichia coli* possess disaggregation activity. In this manner, AAA ATPases can

antagonize proteotoxicity which is resultant from the accumulation of misfolded disease proteins in higher ordered aggregates.[53] In cooperation with HSP70 chaperone systems, the hexameric ring-like complex formed by HSP104 is both capable of resolubilizing protein aggregates as well as restoring the native structure of the resulting monomer.[54] HSP104 activity has been shown to fragment larger amyloid-like prion aggregates into smaller amyloid seeds that enables the faithful transmission of these epigenetic elements to daughter cells in yeast.[55–59] HSP104 action in the disaggregation of amyloid-like fibers and oligomeric intermediates formed by different yeast prions, is dependent on ATP hydrolysis as well as interactions with HSP70 and HSP40 chaperone machinery.[60,61] The aggregation remodeling capabilities of HSP104 strongly influences the ability of the cell to tolerate misfolded disease proteins. With regards to poly-glutamine expansion diseases, HSP104 was able to suppress poly-glutamine aggregation in *Caenorhabditis elegans* and promote survival.[62] Similarly, HPS104 was able to remodel aggregates formed by poly-glutamine expansions in a manner that correlated with suppression of toxicity in a rat model.[63] HSP104 was also able to reduce the formation of oligomeric intermediates and amyloid fibers formed by α-synuclein in a rat model for Parkinson disease.[64] Although HSP104 is a potent suppressor of aggregation and proteotoxicity, it remains to be determined whether humans possess a true HSP104 homolog. Mutations in the mammalian AAA ATPase chaperone, VCP/p97, associated with frontotemporal lobar degeneration (FTLD) with inclusion body myopathy and Paget disease of bone, enable aggregation of disease proteins and pathogenesis.[65] VCP performs a plethora of cellular functions and its role in disease protein detoxification is most likely due to degradation pathways.[66] Yet VCP has been shown to resolublize and refold denatured luciferase[67,68] and its capacity to remodel disease protein aggregates may still play a role in disease progression. Overall, molecular chaperones can intervene early or late in protein aggregation pathways to maintain the solubility of disease proteins and suppress proteotoxicity.

Chaperones Drive the Aggregation of Toxic Proteins

The capacity of the cell to buffer the misfolding of disease proteins can become limited due to chronic insults and age.[27] Under these conditions the cell may no longer be able to maintain disease proteins in a native soluble state. When this occurs molecular chaperones are capable of driving the assembly of disease proteins into tightly ordered aggregates, which appears to reduce the proteotoxic accumulation of soluble oligomeric intermediates. Overexpression of the HSP40, Hdj2, enhances aggregation of an expanded poly-glutamine Huntingtin (Htt), the causative protein for Huntington's disease.[69] Furthermore, purified forms of HSP70 and HSP40 reduced the accumulation of poly-glutamine polypeptides as oligomeric species and promoted their assembly into amyloid-like fibrillar structures.[70] These studies demonstrated the novel function for molecular chaperones as agonists of disease protein aggregation. More recent work has begun to demonstrate the protective effects of chaperone-dependent protein aggregation. HSP60 chaperone proteins form a multi-subunit cage-like structure that is capable of sequestering and refolding nonnative proteins upon rounds of ATP hydrolysis.[71–74] The Hsp60 chaperonin complex was identified as a suppressor of poly-glutamine-mediated toxicity in genomic-wide screen in *C. elegans*.[75] Subsequent studies revealed that the detoxification of the poly-glutamine expanded form of the human glutamine-rich Exon I fragment of Htt by the Hsp60 family member TriC correlated with TriC's ability to suppress the accumulation of soluble low molecular weight oligomers.[31,76,77] Interestingly, TriC cooperated with Hsp70 and Hsp40 to convert proteotoxic 200 kDa oligomers into 500 kDa aggregates.[31] Thus, molecular chaperones are capable of remodeling disease proteins into what appears to be benign higher-molecular aggregates.

The ability of chaperones to drive protective protein aggregation has also been observed in the context of amyloid assembly in a yeast model. The HSP40, Sis1, is essential for yeast viability and performs a variety of functions including protein refolding, protein translocation,

translation initiation and maintenance of the [*RNQ*$^+$]/[*PIN*$^+$] prion conformation.[78–82] The term [*RNQ*$^+$] denotes the amyloid-like prion conformation formed by the Rnq1 protein. Rnq1 is not essential for cell viability and possess no toxic characteristics at endogenous levels. Yet modest overexpression of Rnq1 induces cell death when endogenous Rnq1 is in its amyloid-like [*RNQ*$^+$] prion conformation.[21] It appears that the [*RNQ*$^+$] prion alters the conformation of nascent Rnq1 into a proteotoxic form because cells could tolerate the same level of Rnq1 overexpression in the absence of the [*RNQ*$^+$] prion conformer.[21] Elevating Sis1 levels was able to detoxify excess Rnq1 and surprisingly did so by promoting the assembly of nascent Rnq1 into pre-existing [*RNQ*$^+$] aggregates. Inefficiencies in Sis1-mediated assembly of Rnq1 into amyloid-like [*RNQ*$^+$] aggregates caused by mutations in the chaperone binding region or Sis1 depletion, exacerbated Rnq1 toxicity.[21] These data support the notion that intracellular amyloid formation can provide a protective mechanism because it reduces the accumulation of soluble proteins whose native conformation has been altered via interaction with β-sheet-rich templates such as prions. These data also support the notion that HSP40 and HSP70 chaperones cooperate to facilitate the intracellular assembly of amyloid-like aggregates and thereby protect cells from proteotoxicity. A question that still remains is whether the soluble toxic species identified in a number of disease states represents an on-pathway amyloid assembly intermediate or an off-pathway byproduct caused by inefficient assembly into amyloid-like aggregates.[83] In the case of Rnq1 toxicity, it appears that the accumulation of an off-pathway species contributes to cell death.[21]

DISEASE PROTEIN INERACTION NETWORKS INFLUENCE PROTEOTOXICITY

Molecular chaperones can buffer the interactions of mis-folded disease proteins with the surrounding cellular environment. Yet, chaperones fail to control all aspects of a protein's environment and interactions between disease proteins and neighbors in the crowded cellular milieu strongly impact the folded state of disease proteins. Age or chronic environmental stress may further compromise the availability of chaperone networks and increase the frequency of disease proteins interactions with its surrounding environmental components.[27] A growing body of literature suggests that interactions of disease proteins with other proteins containing similar sequence or structural elements can significantly impact pathogenesis. Yet the mechanism by which these transient protein interactions influence proteotoxicity is unknown.

Thus, there is intense interest in understanding how encounters between disease proteins and other cellular components impact the formation of toxic protein species. An excellent illustration of this phenomenon involves deleterious interactions observed between different, benign glutamine-rich proteins and disease proteins that contain poly-glutamine expansions. Poly-glutamine expansions in a variety of proteins cause dominantly inherited neurodegenerative disorders including Huntington's disease and multiple forms of spinal cerebellar ataxia (SCA). In particular, the SCA2, SCA3, and SCA6 disease states are resultant from expansion within the glutamine-rich domains of the respective cytosolic proteins, Ataxin2, Ataxin3, and CACNA1A (calcium channel subunit). Expansion of the glutamine tract within the Ataxin3 protein results in the SCA3 disease state, yet the normal activity of the unrelated Ataxin2 protein hastens the onset of SCA3 pathology.[84] Additionally, non-pathogenic forms of CACNA1A, a causative gene for SCA6, influences the age onset of a different expansion disease, SCA2.[85] Thus, similarities in primary amino acid sequences enable native proteins within the same subcellular neighborhood to accelerate toxicity of disease-causing proteins.

The ability of glutamine-rich proteins to influence the conformation and toxic nature of otherwise unrelated proteins can also be observed in *Saccharomyces cerevisiae*. The spontaneous conversion of the native prion protein, Sup35, into its amyloid-like [*PSI*$^+$] prion

conformation is hastened by its interactions with heterologous proteins containing similar glutamine and/or asparagine-rich sequences.[86] Yeast possess a number of glutamine-rich proteins which are likely candidates for templating factors.[87] In particular, the yeast prion, Rnq1, is enriched in glutamine and asparagine residues and is required in a conformational specific manner for the glutamine-expanded human Exon I fragment of Htt, Htt-103Q, to become toxic to yeast.[88] In the absence of the amyloid-like [*RNQ*$^{+}$] prion state, Htt-103Q was neither capable of aggregating nor eliciting a toxic affect.[88] Further studies demonstrated that deletion of genes encoding other glutamine-rich proteins can reduce or even eliminate proteotoxicity resultant from the overexpression of Htt-103Q.[89] Subsequent overexpression of these same glutamine-rich proteins could convert Htt-103Q into a toxic species.[89] Thus, this network of unrelated glutamine-rich proteins strongly influences the ability of Htt-103Q to assume a proteotoxic conformation.[88,89] However, it is still not clear how interactions between heterologous proteins can play such a significant role in proteotoxicity and whether such interactions could also provide protective qualities for the cell.

INTERPLAY OF PROTEIN INTERACTION NETWORKS AND MOLECULAR CHAPERONES WITH THE SUBCELLULAR ENVIRONMENT IN CONFORMATIONAL DISORDERS

The subcellular environment which harbors disease-causing proteins has a profound effect on proteotoxicity. Numerous cellular factors present in different cellular locations can contribute to the stability of the disease protein. In particular, distinct chaperone networks exist in most every subcellular organelle and relocation of disease proteins to different compartments enables different sets of molecular chaperones to act on the disease protein. In a like manner, different interacting partners reside in distinct subcellular locations and can influence the folding state of the disease protein. Thus, the subcellular location of the disease protein dictates the molecular chaperones and interacting proteins which come into contact with the disease protein.

It is well established that relocation of disease proteins away from their resident subcompartment is a marker for the disease state. Normal Htt protein contains a 25 residue glutamine sequence within exon I and predominately resides within the cytoplasm.[90] Huntington's disease arises when the poly-glutamine region is expanded beyond 39 residues and the mutant protein becomes enriched within the nucleus of affected neurons.[24,91] In fact, relocation of glutamine-expanded Htt fragments to the nucleus by exogenous nuclear localization signals can exacerbate proteotoxicity.[92] Conversely, the 43-kDa TAR-DNA-binding protein, TDP-43, normally functions within the nucleus. However relocation of TDP-43 out of the nucleus enables the formation of cytoplasmic aggregates in two different disease states including amyotrophic lateral sclerosis (ALS) and frontal temporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U).[25,93] It is not clear how relocation of a disease protein from its normal resident compartment accelerates pathogenesis. Disease proteins relocation may introduce a new and unfavorable set of intermolecular interactions. These interactions might nucleate the formation of the toxic protein species, provide access of the toxic protein species to sensitive targets, and/or hinder detoxification events. Yet recent evidence demonstrates that relocation of the model disease protein, Rnq1, from the cytosol to the nucleus promotes efficient assembly of nascent Rnq1 into benign amyloid-like aggregates and suppresses cell death.[94] In this case, the new subcellular location provides a favorable environment in which more efficient amyloid assembly could occur. Thus, environmental components in different compartments can either accelerate or ameliorate proteotoxicity.

The subcellular environment dictates the protein interaction networks available to the disease protein. However, heterologous protein interactions can, in turn, influence the location of the

disease protein in the cell. Nuclear inclusions formed by the glutamine-expanded Ataxin1 protein were capable of recruiting the unrelated Ataxin3 protein from the cytosol into the same nuclear inclusions.[95] More recent work has begun to link disease protein relocation with proteotoxicity. Targeting glutamine-expanded Htt proteins from the cytosol to the aggresome via a proline-rich aggresome targeting signal[96] correlated with suppression of Htt toxicity in yeast.[97] As an important component in protein quality control machinery, the aggresome is a juxtanuclear compartment comprised of non-native, aggregated proteins that escaped degradation by the ubiquitin-proteasome system or autophagy.[98] Interestingly, Htt-103Q without the proline-rich signal sequence could be sequestered to the aggresome through heterologous interactions with Htt-25Q (shorter, nontoxic glutamine stretch) which contains the aggresome targeting signal.[96] These relocation events correlate with a dramatic suppression of Htt toxicity.[89] Similarly, shifting the site of Rnq1 aggregation from the cytosol to the nucleus sequestered Htt-103Q to the nucleus and dramatically enhanced Htt toxicity.[94] Thus, heterologous interactions between disease proteins and otherwise unrelated components can prompt disease protein relocation and strongly influence proteotoxicity.

The subcellular environment determines the chaperone machinery which can act on the misfolded disease protein. The HSP40 Sis1 has been shown to play an integral role in Rnq1 detoxification[21] and is predominately localized in the nucleus.[78,94] Therefore it seems logical that shifting Rnq1 aggregation pathways to the nucleus could promote protective Rnq1 amyloid assembly and suppress cell death.[94] Similarly, the TriC chaperonin complex antagonizes Htt toxicity[75] and is predominately cytosolic.[99] Thus relocation of the glutamine-expanded Htt protein to the nucleus may hinder detoxification by the TriC chaperonin. It is also feasible that the nuclear enrichment of Htt-103Q may interfere with aggresome targeting and permits accumulation of toxic protein species. Nonetheless, maintenance of disease proteins in a benign or less toxic state correlates with its subcellular accessibility to the appropriate chaperone partners.

CONCLUSION

Neurodegenerative disorders are characterized by the accumulation of misfolded disease proteins in susceptible neurons. Many disease-related proteins are broadly expressed throughout the brain yet it is not clear why particular neuronal subtypes are more vulnerable to proteotoxic misfolding events. Cellular factors involved in regulation of protein homeostasis appear to play a significant role in disease progression. Differential expression of such cellular factors and compartment-specific differences in their activity may provide an explanation for the differential susceptible of neurons to proteotoxicity.

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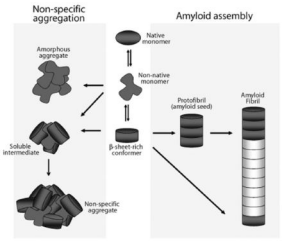

FIGURE 1.

Pathways for the assembly of misfolded proteins into different aggregation states. A native protein can misfold into a nonnative, conformation as a result of mutation or cellular stress. The nonnative monomer is highly unstable and can sample numerous conformations including a β-sheet-rich form. The self-association of the β-sheet-rich monomers can result in a stable protofibril or amyloid seed which can subsequently drive the autocatalytic conversion of protein monomers into the growing amyloid fibril. Alternatively, inefficient flux through amyloid assembly pathways can result in the formation of off-pathway aggregates including amorphous or nonspecific aggregates.