

Protein homeostasis and aging in neurodegeneration

Peter M. Douglas and Andrew Dillin

Howard Hughes Medical Institute, Glenn Center for Aging Research, Molecular and Cell Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037

Genetic and environmental factors responsible for numerous neurodegenerative diseases vary between disorders, yet age remains a universal risk factor. Age-associated decline in protein homeostasis, or proteostasis, enables disease-linked proteins to adopt aberrant tertiary structures, accumulate as higher-ordered aggregates, and cause a myriad of cellular dysfunctions and neuronal death. However, recent findings suggest that the assembly of disease proteins into tightly ordered aggregates can significantly delay proteotoxic onset. Furthermore, manipulation of metabolic pathways through key signaling components extends lifespan, bolsters proteostasis networks, and delays the onset of proteotoxicity. Thus, understanding the relationship between proteostasis and aging has provided important insights into neurodegeneration.

Introduction

The cellular environment is comprised of millions of proteins packed into their respective subcellular locales. Thus, the cell has a daunting task of maintaining proper protein homeostasis, or proteostasis, in the face of intrinsic and environmental stressors. More encompassing than protein quality control, proteostasis involves the global regulation of transcription, translation, folding, trafficking, processing, assembly/disassembly, localization, and degradation (Fig. 1). Thus, the proteostasis concept entails an elaborate and integrated cellular network that governs the “life of proteins” from conception to their demise (Balch et al., 2008). Interplay between proteostasis network components is essential for the long-term health of the cell. Due to the complexity of the integrated network, defects in any one branch can elicit a breakdown of the entire network and manifest themselves in numerous metabolic, oncological, cardiovascular, and neurodegenerative diseases. Thus, a convergence of different biological and biomedical disciplines is critical to reach a thorough understanding of proteostasis in the context of disease.

Correspondence to Andrew Dillin: dillin@salk.edu

Abbreviations used in this paper: APP, amyloid precursor protein; HIF, hypoxia-inducing factor; HSF, heat-shock factor; IGF, insulin-like growth factor; TOR, target of rapamycin.

Activity of Proteostasis Network Hubs

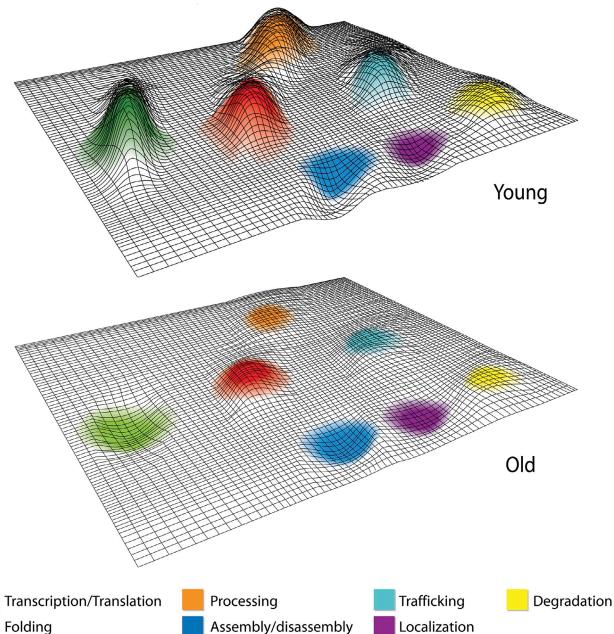


Figure 1. Proteostasis landscape deteriorates with time. Integrated components of the protein homeostasis network are arbitrarily represented as a global landscape. General activity for transcription, translation, folding, trafficking, processing, assembly/disassembly, localization, and degradation are depicted as nodes in which node amplitude signifies strength of homeostatic activity. Nodes are designated with the color indicated in the legend below and assigned an arbitrary value for activity. Proteomic breakdown resultant from age can be attributed to a general deterioration in each proteostasis hub.

Proteostasis network components play critical roles in governing the life of disease proteins. Numerous neurodegenerative diseases are characterized by the misfolding and accumulation of select proteins in insoluble inclusions or aggregates. Clinical onset does not typically occur until middle age or later depending on whether the disease state arises via familial or sporadic means. Thus, neurons can sustain conformationally challenged, disease proteins in benign states for decades until age-associated alterations in cellular stasis predispose different neuronal populations to neurotoxicity.

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Proteostasis declines as a function of time and may provide a feasible explanation as to why age is a major risk factor for such neurodegenerative diseases (Balch et al., 2008). Molecular chaperones and degradation machinery normally act as a first line of defense to solubilize, refold, or degrade misfolded proteins, yet age compromises these protein quality control components (Sherman and Goldberg, 2001; Morimoto, 2008). Thus, aged cells show an increasing inability to maintain metastable proteins in properly folded states (Ben-Zvi et al., 2009). Furthermore, a specific mechanism in the brains of aged individuals causes frame shift mutations at the RNA level and generates nonsense polypeptides, which further burdens the proteome (van Leeuwen et al., 1998). The introduction of metastable, disease-linked proteins can escape protein quality control later in life and initiate a cascade of protein destabilization in the face of deteriorating proteostasis networks (Gidalevitz et al., 2006). Due to the global breakdown of proteostasis with age, cells must manage the increasing burden of protein misfolding without a means of efficiently controlling protein synthesis, processing, localization, refolding, or degradation pathways.

Aging was initially thought to be a random process of deterioration that occurred independent of genetic control (Reichel, 1966). However, evidence for the genetic modulation of aging across the evolutionary scale has silenced old dogma and opened up an era with limitless possibilities for improved health and disease intervention (Kenyon, 2010). Central to the concept of age modulation are the cellular and organismal responses to environmental conditions such as nutrient availability and stress (Fig. 2). Indeed, manipulation of stress response genes and nutrient sensors extends lifespan. Moderate reduction in caloric intake triggers alterations in cellular physiology and mediates lifespan extension and increased resistance to stress (Bishop and Guarente, 2007). The cellular response to dietary restriction is complex and, depending on the food regimen (Mair et al., 2009), requires different signaling pathways including nutrient sensors such as the kinase target of rapamycin (TOR; Kapahi et al., 2004; Kaeberlein et al., 2005b), AMP kinase (Greer et al., 2007), sirtuins (Rogina and Helfand, 2004) and insulin/insulin-like growth factor (IGF-1; Arum et al., 2009; Honjoh et al., 2009) as well as stress response genes such as heat-shock factor 1 (HSF-1; Steinkraus et al., 2008) and hypoxia-inducible factor (HIF-1; Chen et al., 2009). We highlight how different age-extension components modulate proteostasis networks in a manner which protects against proteotoxicity and neurodegeneration.

Proteotoxicity and neurodegeneration

The misfolding and accumulation of disease proteins characterizes numerous late-onset neurodegenerative diseases including Alzheimer's, Huntington's, and Parkinson's disease. Pathogenic states resulting from protein destabilization and inclusion formation are often referred to as conformational diseases. The particular disease protein defines the disorder and can vary in functionality between different diseases, yet pathogenesis may occur by a common mechanism due to the formation of similar amyloid-like inclusions (Carrell and Lomas, 1997). Amyloids are highly ordered, fibrillar aggregates in which exposed β -sheets from aberrantly folded proteins serve as hydrogen bonding

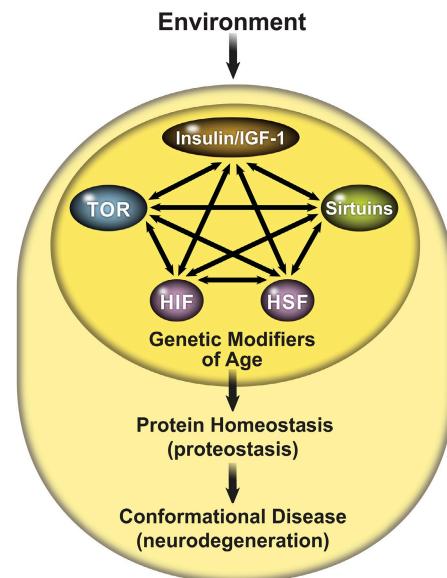


Figure 2. Environment influences neurodegeneration through age modifiers and proteostasis networks. Environmental cues such as food availability or extrinsic stress alter age-modifying, signaling pathways such as insulin/IGF-1, target of rapamycin (TOR), sirtuins, heat shock factor (HSF), and hypoxia-inducible factor (HIF-1). Elaborate interplay between the different signaling pathways modulates proteostasis and impacts onset and progression of numerous neurodegenerative diseases.

partners for other β -sheet-rich proteins and enable polypeptides to pack tightly into fibrous protein structures (Caughey and Lansbury, 2003; Nelson et al., 2005). Many of the amyloid diseases possess a toxic gain-of-function because their aberrant tertiary and quaternary structures trigger pathogenic cascades as opposed to loss-of-function diseases in which removal of essential proteins via misfolding or premature degradation is at the root of pathogenesis. Gain-of-function diseases have been quite perplexing due to the multitude of dysfunctional outputs that include, but are not limited to, transcriptional deregulation, oxidative and membrane damage, mitochondrial injury, aberrant trafficking and signaling, neuroinflammation, and disrupted ion homeostasis (Bossy-Wetzel et al., 2004; Fiala, 2007).

For decades, amyloid deposits formed by disease-linked proteins were considered to be the toxic species due to their overwhelming presence in the brains of postmortem patients. Yet a poor correlation exists between the degree of amyloid accumulation and the clinical severity of the different disease states. In fact, multiple proteins are capable of forming benign amyloid structures that function in phenotypic adaptation in *Saccharomyces cerevisiae* (True et al., 2004; Shorter and Lindquist, 2005) and melanin synthesis in humans (Fowler et al., 2006). These observations prompted researchers to reexamine amyloid assembly pathways in disease. Recent work has begun to challenge the notion that amyloid is indeed the underlying toxic species in conformational diseases and may rather represent a protective mechanism used by cells to ameliorate soluble, toxic intermediates in the amyloid assembly pathway (Fig. 3; Behrends et al., 2006; Cohen et al., 2006; Douglas et al., 2008). Interestingly, the amyloidogenic intermediates formed by multiple disease proteins share similar structures due to recognition by a single

antibody and further bolster the notion for a common mechanism of pathogenesis (Kayed et al., 2003). Thus, a common means for therapeutic intervention may transcend multiple conformational diseases. These paradigm-shifting results have led the field to revise previously held notions of amyloid formation and its role in neurodegeneration.

Alzheimer's disease. Over a century ago, fibrillar structures were discovered within the brains of postmortem patients exhibiting progressive cognitive dysfunction and psychosis by Alois Alzheimer (Alzheimer et al., 1995). Since then, Alzheimer's disease has become the most widely studied neurodegenerative disorder. Numerous heritable mutations in the amyloid precursor protein (APP) have been linked to disease onset during the fifth decade of life, whereas a majority of the Alzheimer's cases are sporadic and develop clinical onset during the seventh decade or later. The disease state arises due to sequential cleavage of APP (Glenner and Wong, 1984) by the β -secretase, β -site APP-cleaving enzyme or BACE1 (Farzan et al., 2000), followed by presenilin-1, a component of the γ -secretase complex (Wolfe et al., 1999). These endoproteolysis events generate A β peptides comprised of 40- and 42-amino acid residues, A β_{40} and A β_{42} , which subsequently assemble into extracellular amyloid plaques (Selkoe, 2004). Autosomal dominant mutations in APP and the presenilins, PS1 and PS2, are responsible for familial forms of Alzheimer's disease and subsequently lead to increased production of the amyloidogenic A β_{42} relative to A β_{40} (Sisodia and St George-Hyslop, 2002). Additionally, patients with APP gene duplications or individuals with Down's syndrome (trisomy 21), who have increased dosage of the APP gene located on chromosome 21, develop early-onset Alzheimer's disease and overproduce A β_{42} (Hardy, 2006). Alternatively, α -secretase cleaves APP within the A β domain and ameliorates A β formation (Weidemann et al., 1989; Esch et al., 1990). In fact, α -secretase processing of APP generates a soluble ectodomain termed APP α , which may possess neuroprotective properties (Postina, 2008). Thus, the proteolytic generation and cellular management of A β_{42} play a central role in Alzheimer's pathogenesis.

Recent findings have begun to challenge the notion that A β plaques are the primary neurotoxic species in Alzheimer's disease but may rather represent the proteinaceous aftermath of neuronal dysfunction or a protective depot for A β aggregation intermediates. Transgenic mouse models have shown that intracellular A β initiates neuronal dysfunction before it accumulates in extracellular plaques (Chui et al., 1999; Hsia et al., 1999; Kumar-Singh et al., 2000). Refocused efforts have demonstrated that soluble, oligomeric intermediates in the A β aggregation pathway are the most toxic entity (Haass and Selkoe, 2007; Shankar et al., 2008). In fact, driving active assembly of A β into amyloid fibrils reduces the concentration of intermediate A β oligomers and functional deficits in tissue culture (Cheng et al., 2007), *Caenorhabditis elegans* (Cohen et al., 2006), and mouse models (Cohen et al., 2009). Finally, transgenic mice expressing a mutant form of A β that enables oligomerization without fibrillization are equally susceptible to Alzheimer's disease-like syndromes as animals expressing A β that can form amyloid (Tomiyama et al., 2010).

Insoluble fibrillar deposits formed by the Tau protein represent the other major pathogenic marker for Alzheimer's disease as well as other diseases characterized by Tau fibrils (termed tauopathies). These neurofibrillary tangles are typically observed within the cell bodies and dendrites of neurons (Kidd, 1963). Particularly enriched in neurons (Binder et al., 1985), Tau acts as a microtubule-stabilizing protein (Drechsel et al., 1992) that is normally phosphorylated at multiple sites. Yet abnormal hyperphosphorylation of Tau reduces its microtubule binding capacity (Alonso et al., 1994) and accelerates aggregation (Alonso et al., 1996). Thus, disruptions in proteostasis networks resultant from age may increase kinase or decrease phosphatase activity, although further investigation is required. Furthermore, other post-translational modifications of Tau may play important roles in regulating its phosphorylation and aggregation status such as O-GlcNAcylation, ubiquitination, sumoylation, and nitration (Liu et al., 2004; Gong et al., 2005).

Neurofibrillary tangles were previously implicated as the key Tau component in Alzheimer's pathogenesis (Arriagada et al., 1992; Bennett et al., 2004). Yet recent studies demonstrate that the formation of neurofibrillary tangles can be uncoupled from cognitive decline and neuronal death (Santacruz et al., 2005). Thus, neurofibrillary tangles may not represent the toxic Tau species, at least in the early stages of pathogenesis. In fact, oligomeric intermediates in the Tau aggregation pathway more closely correlate with the development of functional deficits during disease progression (Berger et al., 2007). In cases of Tau and A β , it appears that intermediates in the amyloid assembly pathway represent the toxic culprit. However, it remains controversial whether A β or Tau is the predominant component behind neuronal dysfunction and death in Alzheimer's disease.

Polyglutamine expansion diseases. CAG nucleotide expansions within a set of unrelated genes are the cause of at least nine different late-onset neurodegenerative diseases, which include Huntington's disease, spinobulbar muscular atrophy, and the spinocerebellar ataxias (Ross, 2002). Huntington's disease represents the most widely studied of these disorders and is classified as a hyperkinetic movement disorder. The disease state arises due to glutamine-encoding expansions in the huntingtin gene, usually beyond a critical threshold of 40 glutamine residues (Zoghbi and Orr, 2000), which results in the accumulation of mutant huntingtin protein within intranuclear inclusion bodies (DiFiglia et al., 1997). However, pathogenesis does not correlate well with the concentration and subcellular distribution of huntingtin inclusions (Saudou et al., 1998; Kuemmerle et al., 1999). In fact, inclusion body formation by mutant huntingtin sequesters soluble forms of the mutant protein and reduces cell death in cultured neurons (Arrasate et al., 2004). Furthermore, inclusion body formation of mutant huntingtin by pharmacological means ameliorates proteosomal dysfunction, a common feature in huntingtin-induced proteotoxicity (Bodner et al., 2006). These data suggest a mechanism for neurotoxicity that likely involves soluble, oligomeric forms of mutant huntingtin rather than higher-ordered inclusions. Indeed, the intracellular accumulation of low-molecular weight oligomers formed by polyglutamine expansions correlated with toxicity in cell culture and yeast (Kitamura et al., 2006). Elevated expression of a potent

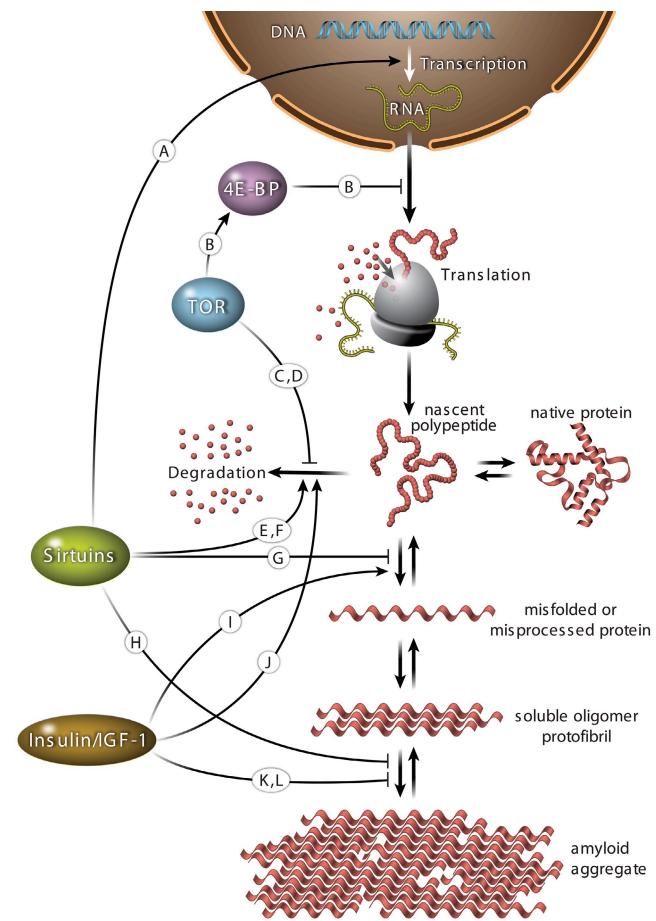


Figure 3. Aging modifiers intervene in the life of disease proteins. The model depicts synthesis, folding, processing, degradation, and aggregation of an arbitrary disease-linked protein. Age modifiers including TOR, sirtuins, and insulin/IGF-1 act at multiple points within the protein aggregation cascade to ameliorate the accumulation of proteotoxic intermediates. The corresponding letters A–L are briefly described and referenced in Table I.

suppressor of polyglutamine toxicity, the TriC chaperonin complex (Nollen et al., 2004), remodeled 200-kD proteotoxic oligomers into benign 500-kD aggregates in a yeast model (Behrends et al., 2006). These data provided some of the first examples by which depletion of toxic oligomers through protective aggregation correlates with disease protein detoxification.

Parkinson's disease. Parkinson's disease is the second most common age-related neurodegenerative disease. The disease is characterized by the accumulation of α -synuclein, a presynaptic protein of unknown function, in Lewy bodies that particularly affect the dopaminergic neurons in the substantia nigra (Dauer and Przedborski, 2003). The majority of Parkinson's disease cases are sporadic, yet mutations have been identified in familial cases of the disease. Single point mutations in the α -synuclein protein (A30P, A53T, and E46K) have been identified in early-onset Parkinson's disease patients (Lotharius and Brundin, 2002; Moore et al., 2005). Additionally, triplication of the α -synuclein gene causes Parkinson's disease in some individuals (Singleton et al., 2003). Elevated expression of the human α -synuclein led to dopaminergic neuronal degeneration and motor deficits in transgenic fly, mice, and rat models (Feany and Bender,

2000; van der Putten et al., 2000; Lo Bianco et al., 2002). Subsequent overexpression of α -synuclein in cell culture led to cytotoxicity in the presence of dopamine (Moussa et al., 2004). These observations have linked α -synuclein dysfunction with Parkinson's disease pathogenesis. Yet the identity of the cytotoxic species formed by α -synuclein remained unclear. Driving large inclusions of α -synuclein by pharmacological means protected against cytotoxicity (Bodner et al., 2006). A major catechin of green tea, (−)-epigallocatechin-3-gallate (EGCG), converted large α -synuclein inclusions into smaller, amorphous protein aggregates that were not toxic to mammalian cells. The mode of action reveals that the compound directly binds to β -sheet-rich α -synuclein aggregates and mediates their conformational change without their disassembly into small diffusible oligomers (Bieschke et al., 2010). Thus, soluble oligomers formed by α -synuclein most likely represent the toxic proteinaceous species and the formation of higher-ordered aggregates, whose nature is still being defined, can reduce the proteotoxic load.

Prion diseases. The infectious protein phenomena underlies multiple transmissible spongiform encephalopathies which include scrapie in sheep and goats, bovine spongiform encephalopathy or mad cow disease, chronic wasting disease in deer and elk, and Creutzfeldt-Jakob disease in humans. Central to prion diseases is the conversion of the prion protein, PrP^C, into the infectious, β -sheet-rich isoform, PrP^{SC}, which drives the autocatalytic conversion of normal PrP^C into the pathogenic PrP^{SC} and enables its accumulation in amyloid aggregates (Prusiner, 1998). PrP^C is a nonessential glycosylphosphatidylinositol-anchored glycoprotein of unknown function that is ubiquitously expressed throughout the brain. Interestingly, PrP^C expression is required for neurotoxicity and prion replication as mice devoid of PrP^C are resistant to infection and disease (Büeler et al., 1993). Multiple models have been proposed for the underlying causes of prion pathogenesis, yet it is still not clear whether neurodegeneration is due to a loss of functional PrP^C or a toxic gain-of-function by PrP^{SC}. Consistent with other neurodegenerative diseases, clinical symptoms can manifest without any obvious scrapie deposits (Collinge et al., 1990; Medori et al., 1992). Rather, soluble oligomers formed by PrP possess higher degrees of infectivity (Silveira et al., 2005) and neurotoxicity (Kazlauskaitė et al., 2005; Simoneau et al., 2007) than the higher-ordered amyloid fibrils. Thus, prion diseases adhere to the common trend whereby soluble oligomers formed by disease-linked proteins represent the most toxic entity. Amyloid deposition may reduce oligomeric concentration and delay proteotoxic onset.

Genetic modifiers of age protect against neurodegeneration

Proteostasis networks buffer the constant flux of protein misfolding caused by the inherently error-prone nature of protein synthesis and degradation. Yet proteostasis networks deteriorate as a function of time and make cells more vulnerable to proteotoxic stress. Genetic mutations within conformational disease proteins and environmental stress can further increase the burden of destabilized proteins and cause irreversible damage to aged cells. However, manipulating the aging process through key metabolic signaling molecules can hamper age-mediated proteostasis

Table I. Age modifiers and neurodegeneration

	Disease Protein	Disease	Age modifier	Organism	Reference
A	PrP	Prion diseases	SIRT1 knockout	Mouse	Chen et al., 2008
B	α -Synuclein	Parkinson's disease	Rapamycin inhibition of TOR and 4E-BP overexpression	<i>Drosophila</i>	Tain et al., 2009
C	Polyglutamine expansion, Tau	Polyglutamine-expansion diseases, Alzheimer's disease	Rapamycin inhibition of TOR	Tissue culture and <i>Drosophila</i>	Berger et al., 2006
D	Polyglutamine expansion	Polyglutamine-expansion diseases	Rapamycin inhibition of TOR	Tissue culture, <i>Drosophila</i> , mice, and human	Ravikumar et al., 2004
E	A β	Alzheimer's disease	Resveratrol activation of Sirtuin	Cell culture	Marambaud et al., 2005
F	—	—	Deletion and overexpression of SIRT1	Mouse	Lee et al., 2008
G	A β	Alzheimer's disease	SIRT1 overexpression	Mouse	Qin et al., 2006b
H	α -Synuclein	Parkinson's disease	SIRT2 inhibition	Cultured neurons and <i>Drosophila</i>	Outeiro et al., 2007
I	A β	Alzheimer's disease	IRS-2, neuronal IGF-1R and insulin receptor knockout	Mouse	Freude et al., 2009
J	A β	Alzheimer's disease	IGF-1 overexpression	Mouse	Palazzolo et al., 2009
K	A β	Alzheimer's disease	DAF-2 reduction	<i>C. elegans</i>	Cohen et al., 2006
L	A β	Alzheimer's disease	IGF-1R reduction	Mouse	Cohen et al., 2009

References and descriptions for Fig. 3. Each letter highlights its respective arrow in Fig. 3 and references the corresponding study.

collapse and significantly delay the onset of neurodegeneration caused by conformationally challenged, disease proteins. These age-extension components act at multiple stages in the misfolding and aggregation pathway to inhibit transcription or translation, reduce aberrant processing, and promote disease protein refolding, degradation, or active aggregation (Fig. 3 and Table I).

Dietary restriction. Caloric or dietary restriction represents one of the most studied phenomena known to extend lifespan. Organisms ranging from single-cell eukaryotes to mammals balance growth and reproduction with environmental stress and nutrient availability. Under optimal conditions with ample food, organisms allocate energy expenditure in favor of growth, development, and reproduction. Yet challenging conditions or limited food availability elicit global physiological responses that divert energy into protective pathways, assist in proper cellular stasis, and can also extend lifespan. Moderate decrease in calorie intake without malnutrition extends lifespan in yeast, flies, worms, mice, and primates (Colman et al., 2009). Dietary restriction has also been shown in multiple disease models to attenuate proteotoxicity caused by different conformational disease proteins.

Dietary restriction can significantly delay the development of spontaneous age-related changes in the brain (Finch and Cohen, 1997). For instance, dietary restriction suppresses age-related decreases in synaptic spine density in the neocortex of rats (Moroi-Fetters et al., 1989) and significantly reduces the age-related degeneration of spiral ganglion auditory neurons in mice (Park et al., 1990). The protective aspects of dietary restriction on cellular health extend to different disease models. Dietary restriction delays the proteotoxic onset caused by different conformational disease proteins (polyglutamine expansions and

amyloidogenic A β -peptides) in a nematode model (Steinkraus et al., 2008). Human studies indicate that increased caloric intake may accelerate Alzheimer's disease pathogenesis (Hendrie et al., 2001; Luchsinger et al., 2002). Subsequent analysis of Alzheimer's disease models in mice revealed that restricted food regimens prevented the generation of A β peptides and plaque deposition in mouse brains through increases in anti-amyloidogenic α -secretase activity (Wang et al., 2005). Similarly, reductions of calorie intake in squirrel monkeys showed a dramatic decrease in the steady-state levels of amyloidogenic A β , which correlated with increased α -secretase levels but not the A β peptide generating γ - and β -secretases (Qin et al., 2006a). Dietary restriction in adult mice also increases resistance of striatal dopaminergic neurons to the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in a Parkinson's disease model (Duan and Mattson, 1999). Thus, dietary restriction attenuates the deleterious effects of neurodegeneration in multiple disease models.

Recent research has begun to identify key molecular components required for dietary restriction-mediated protection against disease protein misfolding and aggregation. Although dietary restriction has been well established as an age-extension phenomena, relatively little is known regarding the mechanism of action. Genetic analyses have linked multiple age-extension components including the kinase TOR, AMP kinase, HSF-1, HIF-1, sirtuins, and insulin/IGF-1 signaling to dietary restriction. However, these results seem somewhat variable and occasionally opposing. Inconsistencies between different research groups may be attributed to the complexity of the dietary restriction response and method by which dietary restriction is administered in the different model organisms (Mair et al., 2009).

Regardless, age-extension components, which may or may not regulate dietary restriction–induced longevity, intervene at multiple steps in the disease protein misfolding and aggregation pathway to delay the onset of proteotoxicity.

Sirtuin. The sirtuin family of deacetylases has become one of the widest studied components in aging and age-related diseases. The identification of multiple pharmacological activators of sirtuins, including the widely studied resveratrol, has accelerated our understanding of sirtuin’s role in aging and neurodegeneration. The high conservation of sirtuins across the evolutionary scale has also enabled researchers to pool knowledge from multiple model organisms. Yet diversity in the subcellular localization and function of different sirtuin isoforms has made it difficult to discern their individual contributions in a vast array of cellular processes. Sirtuins were initially identified in yeast as silent information regulators (SIRs; Rine and Herskowitz, 1987) that mediate deacetylation of both histone and nonhistone targets. Mammals possess seven sirtuin paralogues with SIRT1 being the closest to the yeast Sir2 (Haigis and Guarente, 2006). The role of sirtuins in aging was first discovered in *S. cerevisiae* as depletion of Sir2 shortened replicative lifespan, whereas overexpression lengthened replicative lifespan (Kaeberlein et al., 1999).

Sirtuins have since been linked to longevity mediated by dietary restriction. Increased replicative lifespan caused by reduction in glucose availability depends on the presence of the SIR2 gene in yeast (Lin et al., 2000). Yet the involvement of sirtuins in longevity mediated by dietary restriction remains controversial. Replicative lifespan extension induced by glucose reduction is independent of Sir2 activity under certain conditions (Kaeberlein et al., 2004, 2005a). Consistent with the complexity of the dietary restriction response, the role of sirtuins in mediating age extension appears variable and depends on the specific dietary restriction conditions. In *C. elegans*, sir-2.1–mediated lifespan extension is dependent on the FOXO transcription factor DAF-16 (Tissenbaum and Guarente, 2001) and interactions with the 14-3-3 proteins (Berdichevsky et al., 2006). However, discerning the role of sirtuins in lifespan extension has yielded conflicting results. In one study, resveratrol treatments extended worm and fly lifespan in a Sir2 orthologue–dependent manner (Wood et al., 2004). Alternatively, resveratrol treatment had no effect on fly lifespan and variable results on worm lifespan (Bass et al., 2007). In a mouse model, resveratrol lengthened the lifespan of mice fed a high calorie diet (Baur et al., 2006) but had no effect on the lifespan of mice fed a standard diet (Pearson et al., 2008). Despite conflicting findings regarding pharmacological analysis, the ability of Sir2 orthologues to promote longevity extends across the evolutionary scale, yet it remains unclear whether age extension through dietary restriction requires the activity of sirtuins.

Sirtuin’s beneficial role in the aging process has made it an attractive target in age-related neurodegenerative diseases. Resveratrol protected *C. elegans* and mouse neurons against mutant huntingtin-induced cytotoxicity (Parker et al., 2005). Additionally, increased sir-2.1 gene dosage in the worm model of Huntington’s disease also protected against neurotoxicity in a DAF-16–dependent manner. Subsequently, depletion of sir-2.1 accelerated age-dependent aggregation of α -synuclein

in transgenic worms, yet its effect on proteotoxicity and lifespan were not examined in this study (van Ham et al., 2008). Resveratrol also antagonized degeneration of cultured neurons caused by the neurotoxin 1-methyl-4-phenylpyridinium (MPP), which selectively targets dopaminergic neurons and is a widely used agent to mimic Parkinson’s disease in rodents (Alvira et al., 2007). In contrast, flies appear to show opposing effects whereby reductions in the dSir2 activity by genetic and pharmacological means promoted neuronal survival in mutant huntingtin transgenic flies (Pallos et al., 2008). Similarly, SIRT1 knockout mice exhibited delayed onset of prion disease (Chen et al., 2008). Thus, activation and inhibition of Sir2 orthologues drastically affects proteotoxicity. However, these effects are dependent on the disease protein and model organism.

The ability of Sir2 orthologues to modulate lifespan and proteotoxicity prompted researchers to examine the mechanistic links of SIRT1’s role in age and proteome maintenance. Activation of SIRT1 positively regulates the heat-shock factor, HSF-1 (Westerheide et al., 2009). HSF-1 extends lifespan in worms (Hsu et al., 2003; Morley and Morimoto, 2004) and regulates the expression of numerous molecular chaperones that are potent modulators of protein aggregation and neurodegeneration (Muchowski and Wacker, 2005; Douglas et al., 2009). Thus, the ability of SIRT1 to maintain proteome stability and protect against proteotoxicity may act, at least in part, through the activation of the stress response gene HSF-1.

The ability of sirtuins to modulate proteostasis enables it to intervene at multiple stages in the disease protein aggregation pathway and delay proteotoxic onset. It can act early in the disease protein aggregation pathway to ameliorate flux of disease proteins through the cell. SIRT1 knockout as well as dietary restriction in a mouse model reduced PrP mRNA and protein levels, which correlated with delayed prion disease onset (Fig. 3 A; Chen et al., 2008). Additionally, dietary restriction exerted no further protection on SIRT1 knockout mice, which further suggests that the dietary restriction cascade acts through SIRT1. We previously discussed that dietary restriction can promote the anti-amyloidogenic activity of α -secretase in Alzheimer’s disease models and reduce the production of pathogenic A β . In a similar manner, elevated expression of SIRT1 in mammalian neurons prevented A β production via altered cleavage of APP by α -secretase (Fig. 3 G; Qin et al., 2006b). It remains unclear why removal or overexpression of the same sirtuin isoform, SIRT1, can intervene at different point in the disease protein aggregation pathway and protect against proteotoxicity. The most likely explanation involves distinct roles for SIRT1 in Alzheimer’s and prion disease. However, further study is required to better address this conundrum. Nonetheless, modulating Sir2 orthologue levels can decrease synthesis and aberrant processing of different disease proteins and ultimately reduce proteotoxic flux through the cell.

Sirtuins can also act later in the aggregation cascade to promote removal of toxic oligomeric intermediates formed by disease-linked proteins. As sirtuins can promote favorable processing pathways for APP, they can also promote degradation of the amyloidogenic A β peptide potentially through both major branches of the protein degradation network. Activation of sirtuins by resveratrol reduced A β levels by promoting degradation

of A β via the proteasome system (Fig. 3 E; Marambaud et al., 2005). SIRT1 also deacetylates autophagy genes and stimulates basal autophagy (Fig. 3 F; Lee et al., 2008), which may play a role in clearance of A β and other conformationally challenged proteins. Pharmacological inhibition of SIRT2 drove the formation of large and less toxic α -synuclein aggregates in the cellular model for Parkinson disease and reduced neuronal deficits in culture and in a *Drosophila* model of Parkinson's disease (Fig. 3 H; Outeiro et al., 2007). Thus, it has become apparent that different sirtuin members have very distinct roles in protein detoxification and it is not entirely clear how activation of SIRT1 and inhibition of SIRT2 both ameliorate proteotoxicity caused by disease protein aggregation. Differences in substrate recognition and subcellular localization between the two sirtuin isoforms provide vague yet plausible explanations to this question and will require more intensive investigation to elucidate the molecular mechanisms underlying these observations.

Insulin signaling. The insulin/IGF signaling pathway regulates stress resistance and the aging process. Reductions in insulin/IGF signaling yield stress-resistant, long lived worms (Kenyon et al., 1993), flies (Tatar et al., 2001), and mice (Blüher et al., 2003; Holzenberger et al., 2003). The signal transduction pathway has been well characterized in the nematode *C. elegans*. The homologue of insulin/IGF-1 receptor, DAF-2, is a tyrosine kinase that binds to an insulin-like molecule, activates a phosphatidylinositol-3 kinase, AGE-1, and ultimately results in the phosphorylation and down-regulation of the FOXO transcription factor, DAF-16 (Rincon et al., 2004). Manipulating any aspect of the insulin/IGF-1 cascade can result in stress-resistant, long-lived worms. The insulin/IGF-1 signaling pathway also plays a major role in the detoxification of model disease proteins as reduced signaling enables worms and mice to sustain proteotoxic insult caused by polyglutamine-expansions (Morley et al., 2002) and amyloidogenic A β -peptides (Cohen et al., 2006, 2009). Insulin/IGF-1 signaling accomplishes disease protein detoxification by intervening at multiple points within the aggregation cascade.

Reductions in insulin/IGF-1 signaling act early in the disease protein aggregation pathway to protect against proteotoxicity. In a *C. elegans* model for polyglutamine expansion disease, reduced insulin/IGF-1 signaling by mutations within *age-1* (PI3K) significantly delayed polyglutamine aggregation and proteotoxicity (Morley et al., 2002). Protection by reduced insulin/IGF-1 was dependent on DAF-16. Yet it remains unclear whether reduced insulin/IGF-1 signaling acts at the level of protein synthesis or maintenance of polyglutamine solubility. In a mouse model of Alzheimer's disease, deficiencies in IRS-2, neuronal IGF-1R, or neuronal insulin receptor delayed mortality and decreased A β accumulation by reduced amyloidogenic processing of APP (Fig. 3 I; Freude et al., 2009). Furthermore, IRS-2 null mice exhibited reductions in A β accumulation and behavioral deficits (Killick et al., 2009). Thus, reduced insulin/IGF-1 signaling can act at the level of protein processing to delay the production of toxic proteinaceous species.

Other studies have implicated insulin/IGF-1 signaling as acting later in the disease protein aggregation pathway to reduce the cellular concentration of toxic oligomers. Elevated expression

of IGF-1 in culture reduced aggregation of the androgen receptor (AR), a polyglutamine-expanded protein underlying spinal and bulbar muscular atrophy (SBMA), and increased AR proteosomal clearance through phosphorylation of AR by the PI3K/AKT pathway (Fig. 3 J; Palazzolo et al., 2009). Interestingly, SBMA is a neurodegenerative disease yet overexpression of IGF-1 in skeletal muscle rescued behavior and aberrant histopathology. Alternatively, reductions in insulin/IGF-1 signaling can promote the ordered assembly of A β peptides into amyloid aggregates. In a *C. elegans* model, knockdown of *daf-2* expression delayed nematode paralysis caused by ectopic expression of the A β peptide and promoted A β aggregation, which correlated with decreased concentrations of low-molecular weight A β oligomers (Fig. 3 K; Cohen et al., 2006). This phenomenon was extended into mouse models of Alzheimer's disease. Alzheimer's mice heterozygous for the IGF-1 receptor exhibited reductions in insulin/IGF-1 signaling and delayed the onset of disease symptoms including behavioral impairment, neuroinflammation, and neuronal loss (Fig. 3 L; Cohen et al., 2009). Histopathological analysis of mice revealed that reductions in IGF-1 gene dosage promoted the formation of densely packed A β aggregates, which resulted in decreased steady-state levels of the soluble A β oligomers. These observations have provided compelling evidence for the protective effects of amyloid formation as a means of ameliorating the cellular concentration of neurotoxic oligomers. Indeed, disease protein aggregates are space-filling lesions that may affect later stages of disease pathogenesis. Thus, cells may use aggregation pathways as a secondary means of disease protein detoxification when primary methods of solubilization and degradation have become compromised as a result of increasing age and proteostasis collapse.

TOR signaling. The TOR signaling pathway has been the most consistently linked to dietary restriction (Kenyon, 2010). The TOR kinase monitors intra- and extracellular availability of amino acids and nutrients. In the presence of ample food, TOR stimulates growth and inhibits salvage pathways such as autophagy (Noda and Ohsumi, 1998). Inhibition of the TOR signal transduction pathway by reduced nutrient availability or rapamycin treatment increases resistance to environmental stress (Hansen et al., 2007) and extends the lifespan of yeast (Kaeberlein et al., 2005b), worms (Vellai et al., 2003), flies (Kapahi et al., 2004), and mice (Harrison et al., 2009). Unlike other lifespan extension pathways, TOR signaling does not require the activity of FOXO transcription factor DAF-16, but rather acts through the FOXA transcription factor PHA-4 in worms, which also mediates dietary restriction-induced lifespan extension in worms (Panowski et al., 2007). Consistent with the physiological shift toward tissue maintenance during nutrient deprivation, inhibition of TOR signaling suppresses translation by inhibiting the ribosomal subunit S6 (Ma and Blenis, 2009) and activating 4E-BP, a translation repressor (Brunn et al., 1997). Manipulation of translational regulators downstream of TOR such as 4E-BP, S6 kinase, and translation initiation factors (eIF) modulates lifespan across the evolutionary scale (Evans et al., 2010). Furthermore, physiological changes resultant from TOR signaling inhibition can also protect against proteotoxic stress caused by disease protein aggregation.

Reductions in TOR signaling influence early events in the aggregation cascade by reducing the flux of disease proteins

through the cell. With reductions in food available sensed by TOR, organisms must reduce protein synthesis through the ribosomal subunit S6 and 4E-BP and reallocate available materials through autophagy. The cellular processes dealing with limited nutrients decrease the load of newly synthesized disease protein as well as promote their removal from the cell. Indeed, over-expression of 4E-BP suppressed Parkinson's disease phenotypes including degeneration of dopaminergic neurons in flies (Fig. 3 B; Tain et al., 2009). Furthermore, similar protective results were observed upon rapamycin treatment. Interestingly, protection by 4E-BP activation was not dependent on autophagy, which suggests that repression of translation alone is sufficient to reduce the flux of α -synuclein through the cell. Yet further analysis is required to confirm this hypothesis.

Perhaps better characterized is TOR's ability to act downstream in the disease protein aggregation cascade through autophagy regulation. Conditional knockout of autophagy genes can cause neurodegeneration in the absence of disease mutations (Hara et al., 2006). Thus, perturbations in this branch of the proteostasis network can be detrimental even without genetic predisposition to a disease state. Multiple disease-linked proteins are degraded through autophagy including polyglutamine and polyalanine expansions (Ravikumar et al., 2002), α -synuclein (Webb et al., 2003), and tau (Berger et al., 2006). TOR inhibition via rapamycin treatment induced autophagy and reduced proteotoxicity caused by polyglutamine-expanded huntingtin in fly and mouse models (Fig. 3 D; Ravikumar et al., 2004). Subsequently, a pharmacological inhibitor of TOR, CCI-779, enhanced clearance of mutant huntingtin in cultured cells. Interestingly, mutant huntingtin inclusions sequestered TOR, which suggests a protective feedback loop through TOR inactivation. Subsequent work from the Rubensztein laboratory expanded the range of model disease proteins including mutant tau, which are degraded via rapamycin-induced autophagy and prolonged survival (Fig. 3 C; Berger et al., 2006). Furthermore, genetic inhibition of TOR or induction of autophagy suppressed cell death in fly models of Huntington's disease and phospholipase C (norpA)-mediated retinal degeneration (Wang et al., 2009). Thus, age extension through TOR inactivation bolsters proteostasis clearance hubs and helps maintain folding homeostasis within the cell. Interestingly, aggregate formation sequesters TOR, which may promote autophagic removal of toxic oligomers and represent another protective aspect of disease protein aggregation.

Conclusions

The maintenance of a healthy proteome is essential in the fight against neurodegeneration and aging. Cells are capable of suppressing proteotoxic stress caused by metastable disease-linked proteins for decades. However, a declining proteostasis network accompanies old age and initiates a seemingly irreversible process of disease protein aggregation and cell death. Genetic regulators of age such as sirtuins, HSF-1, insulin/IGF-1, and TOR are capable of prolonging the health of the proteome and further delaying proteotoxic onset by intervening at different points in the disease-protein aggregation cascade. These major age-extension components do not exclusively represent the arsenal by which aging cells combat proteotoxicity. HIF-1 has been shown to extend

lifespan in worms and suppress proteotoxicity (Chen et al., 2009; Mehta et al., 2009), yet its mechanism of action in the disease-protein aggregation cascade has not yet been defined. Through the earlier part of life, cells antagonize disease protein aggregation by reducing disease protein flux via reduced protein synthesis, favorable protein processing, and increased folding and degradation. However, age-mediated deterioration in these proteostasis network hubs forces the cell to resort to secondary means of proteotoxic clearance, which include the ordered assembly of toxic oligomers into benign or less-toxic amyloid aggregates. The protective aspects of amyloid formation are paradigm shifting and have redirected research to identify factors that actively promote aggregation and investigate the dynamics between primary and secondary detoxification mechanisms.

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