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A cellular perspective on conformational disease: The role of genetic background and proteostasis networks

Tali Gidalevitz, Elise A. Kikis, and Richard I. Morimoto

Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University; Rice Institute for Biomedical Research; Evanston, IL 60208-3500, USA

Summary

The inherently error-prone nature of protein biosynthesis and turnover leads to a constant flux of destabilized proteins. Genetic mutations in conformational disease-associated proteins, as well as exposure to acute and chronic proteotoxic stresses, further increase the load of misfolded protein on the proteostasis network. During aging, this leads to enhanced instability of the proteome, failure to buffer destabilizing genetic mutations or polymorphisms, and cellular decline. The combination of cell-type-specific differences in the buffering capacity of the proteostasis network and destabilizing polymorphisms in the genetic background may account for some of the cell-type specificity observed in disease, even when the predominant disease-associated protein is widely expressed.

Introduction

Protein misfolding and aggregation underlies disease

Protein misfolding is recognized as the basis of numerous human diseases, known collectively as conformational diseases [1], including neurodegenerative diseases, certain cancers, type II diabetes and other metabolic pathologies, and various amyloidoses. In the cellular context, misfolding of a protein may lead to aberrant protein and membrane interactions, mislocalization, degradation, and aggregation, ultimately resulting in decreased availability of functional protein, as in Cystic Fibrosis, or gain-of-function toxicity, as in neurodegenerative diseases. Many neurodegenerative diseases, including Alzheimer's Disease (AD), Parkinson's Disease (PD), Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease (HD), and prion diseases, are characterized by the appearance of protein deposits, aggregates, or plaques in the postmortem tissues of affected individuals [2]. Although the identities of proteins involved are distinct, and corresponding diseases have different clinical manifestations, they have certain features suggestive of a common underlying mechanism of toxicity [3]. For example, all have delayed onset, with symptoms appearing usually in late adulthood. Most, with the exception of the polyglutamine diseases, such as HD, have both hereditary and sporadic forms, all cause cellular dysfunction and degeneration, and all are associated with protein misfolding and aggregation. Whether protein aggregation is causally linked to the pathogenesis of these conditions has been a

Corresponding author: Richard I. Morimoto, Department of Biochemistry, Molecular Biology, and Cell Biology, Rice Institute for Biomedical Research, 2205 Tech Drive, Hogan 2-100, Northwestern University, Evanston, IL 60208, Phone: 847-491-3340, Fax: 847-491-4461, r-morimoto@northwestern.edu.

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focus of intense investigation, with much work done both from biophysical and biochemical perspective, and in cell culture and animal models.

Protein deposits associated with disease can be either extracellular or intracellular. Extracellular deposits usually consist of amyloid fibrils and are present in diseases such as light chain amyloidosis, senile systemic amyloidosis, type II diabetes, and familial cases of AD. Intracellular deposits - inclusion bodies or aggregates - can be ordered amyloid structures, non-amyloid fibrils, or amorphous or disordered aggregates. The currently favored hypothesis is that, during aggregation, certain molecular species resulting from conformational conversion of the native protein into non-native, often β -sheet-rich, forms initiate toxic events that ultimately lead to cellular dysfunction and degeneration [4]. While amyloid fibrils or larger, visible, aggregates were considered to be toxic at some point, the misfolded oligomeric forms, soluble oligomers, protofibrils, and even some monomeric species are currently thought of as toxic species [4–7]. This view is supported by the evidence that various non-disease-associated proteins become cytotoxic by conversion to β -sheet-rich oligomers [3]. Membrane disruption and depolarization, induction of oxidative stress, inappropriate activation of signaling pathways, and aberrant intracellular interactions, including partitioning of molecular chaperones and other cellular proteins into aggregates, have been proposed to explain the toxicity of these species [3,8,9]. However, despite much progress, certain aspects of conformational disease remain difficult to reconcile.

An intriguing aspect of neurodegenerative diseases is cell-type-selectivity with respect to the cells and tissues that undergo degeneration in different diseases. This selectivity occurs despite the fact that disease-associated proteins are expressed in a wide range of neuronal and non-neuronal tissues, often at much higher concentrations than in specific neuronal subtypes that are targeted in disease [10–12]. Perhaps consistent with this, in animal models, the disease-associated proteins are often expressed at high levels, and, in the case of polyQ-containing proteins, have longer polyQ lengths than are found in patients. Additionally, sensitized backgrounds have been used to expose their toxicity [13,14]. It appears that the aggregation and toxicity of these proteins depends on the cellular environment in which they are expressed.

Another characteristic aspect of disease is the delayed onset of aggregation and toxicity. Studies in animal models show that the age of onset of aggregation can be modulated genetically, by aging-signaling pathways, by enhancing the expression levels of molecular chaperones, and by activating stress-responsive pathways [15,16 Mojsilovic-Petrovic, 2009 #4711]. Moreover, in mouse models, genetic background has the capacity to override the toxic effects of the mutant protein [17–19]. There is also a large variability in the time of disease onset in patients, for example, in people harboring identical mutations in SOD1, or HD patients with identical or very similar near-threshold lengths of polyQ tract (the genetic determinants of disease) [20–23]. This implies that intrinsic misfolding, or aggregation propensity alone, is not sufficient to explain the initiation of toxicity by the disease-associated protein, and that the genetic background exerts a strong modulatory effect on aggregation and toxicity and therefore on disease severity and the age of onset.

Can some of the lessons learned from animal models of neurodegenerative diseases help refine our understanding of the underlying common mechanism(s) of toxicity? The questions that can be addressed by *in vivo* studies are those that look beyond the intrinsic structural properties of the disease-associated proteins, and consider their cellular context.

Proteostasis networks

One way in which disease-associated aggregation-prone proteins interact with their cellular environment is via the proteostasis networks. “Proteostasis” is, by our definition, the state of

dynamic equilibrium in which protein synthesis and folding is balanced with degradation, while allowing for the conformational flexibility necessary for function, thus leading to a “healthy proteome” [24]. Genetic modifiers of aggregation and/or toxicity provide an insight into the nature of the interaction between the disease-associated, aggregation-prone proteins and the proteostasis networks. Molecular chaperones and the components of the degradation machinery are among the strongest and most consistent modifiers, suggesting that assistance in folding, prevention of aggregation and the disposal of the misfolded and aggregated species are crucial *in vivo*. Some of the modifiers identified in unbiased genetic screens in animal models point to a potential function of the affected proteins, for example genes involved in vesicular trafficking being identified as modifiers of α -syn toxicity [25]. Importantly, the hits obtained by screening for suppressors of polyQ or SOD1 aggregation in *C. elegans* showed a significant overlap with genes that are involved in the response to the osmotic stress-induced protein damage, while many suppressors of polyQ-expanded ataxin-3 in *Drosophila* also suppressed toxicity due to the generic increase in protein misfolding in a HSP70 hypomorphic strain [26–29]. These hits included proteins involved in RNA processing, protein synthesis, protein folding, and degradation, suggesting that a core set of factors function both in response to general stress-induced protein damage, and in response to the expression of disease-associated proteins. This core set of factors, representing the core of the proteostasis networks, may become compromised under conditions of disease and/or aging. The late onset and cell-type-restricted toxicity in conformational disease, despite often ubiquitous expression of disease-associated proteins, strongly support this view.

Proteostasis networks and aging

The aging-related decline in the functionality of proteostasis networks is well documented, and the levels of functional chaperones and the capacity of the clearance mechanisms are affected during aging. Increased protein damage, transcriptional and translational dysregulation, aberrant signaling, and other changes also accompany this decline. These aging-related changes could result in local shifts in the equilibrium between properly folded and misfolded or unfolded states. In this scenario, the susceptibility to conformational disease could be imparted by the cell-type-specific differences in the composition of proteostasis networks and in the robustness of adaptive stress responses.

The molecular interactions between the genetic pathways regulating lifespan and proteostasis are mediated, in part, by factors that detect and respond to misfolded proteins. These factors include molecular chaperones, the heat-shock transcription factor HSF1, which is activated when rapid increase in protein misfolding is induced, the FOXO transcription factor DAF-16, which is regulated by the ILS pathway, and other stress-inducible transcription factors. Downregulation of HSF1 activity in *C. elegans* suppresses the ILS-mediated lifespan extension and protection against proteotoxicity. It also leads to decreased normal lifespan and an accelerated aging phenotype, while overexpression of HSF1 extends lifespan [15,30]. Moreover, both HSF1 and DAF-16 are regulated by the NAD-dependent sirtuin, SIRT1, providing further evidence linking these stress-inducible transcription factors to aging and to the metabolic state of the cell [31–33]. The functional relationship between ILS and protein folding homeostasis can be demonstrated by the induction of both thermotolerance and life span extension either by mutations in the ILS pathway, or by a sublethal heat stress [34]. Furthermore, cells from naturally long-lived [35] or lifespan mutant [36] rodents appear to be resistant to multiple proteotoxic stresses. At the cellular level, the transcriptional output of aging signaling pathways and stress responsive pathways converge on common components of proteostasis networks involved in folding, clearance and detoxification processes, as well as metabolic components.

The integration of proteostasis and aging networks in conformational diseases is underscored by a decrease in aggregation/toxicity of disease-associated proteins upon overexpression of individual molecular chaperones [37], or upon activation of HSF1 or DAF-16. Likewise, proteasomal adaptation (by modulation of substrate accessibility to the proteasome core) to environmental stress in *C. elegans* ensures both resistance to proteotoxic conditions, including polyQ aggregation, and maintenance of life span under normal conditions, arguably through regulating degradation of misfolded proteins [38]. We are only beginning to understand how organismal regulation of growth, metabolism and aging is integrated with the maintenance of proteostasis at the cellular level. For example, recent evidence from *C. elegans* shows that the ability of individual cells in an organism to respond to the proteotoxic conditions is controlled by the activity of a subset of neurons [39].

Proteostasis is not robust

Heat shock proteins (HSPs) function as molecular chaperones in the absence of stress by guiding conformational transitions during synthesis, folding, translocation, assembly, and degradation of proteins [40–42]. Under conditions of stress, such as heat shock, HSPs are upregulated to counteract the deleterious consequences of protein misfolding. The ability of the cell to manage widespread protein damage during these proteotoxic stress conditions is often taken to indicate that the abundant expression of chaperones under basal conditions [43,44], together with the adaptive stress responses, provides sufficient ‘folding capacity’ to buffer unexpected folding requirements. However, the accumulation of misfolded and aggregated proteins in aging-related conformational diseases, and the associated toxicity, challenge this view and indicate a failure of these pathways.

Several possible, not mutually exclusive, explanations may account for such failure. First, HSPs levels or activity are often not enhanced in symptomatic cells, despite the accumulation of misfolded and aggregated proteins [45]. For example, in *C. elegans*, intracellular aggregation of polyQ proteins only sporadically activated HSP expression [46]. This is consistent either with the idea of an organismal override of the cell-autonomous stress responses [39], or with the accumulation of misfolded proteins in conformational diseases being too gradual, or not reaching the threshold necessary for the activation of the heat shock response. Second, molecular chaperones, components of degradative machinery and other proteins are often found trapped in aggregates, potentially mimicking hypomorphic phenotypes [46–49]. Third, there is evidence that expression of disease-associated misfolded proteins can interfere with key components of the proteostasis network such as the proteasome [50,51], and inhibits the heat shock response. This could potentially indicate that cells (or organisms) can adapt to the chronic expression of misfolded proteins by actively preventing stress induction. Finally, stress responses may simply fail to adequately address chronic misfolding. For example, a mouse *sti* mutation in the tyrosyl-tRNA synthetase, a model for a subtype of Charcot-Marie-Tooth neuropathy [52], leads to the production of heterogeneous misfolded proteins, accompanied by increased expression of chaperones in the cytoplasm and the endoplasmic reticulum (ER) [53]. Although an observed increase in chaperone expression suggests that adaptive transcriptional responses are indeed activated, the cellular dysfunction and neurodegeneration in this mouse model indicate that chronic protein misfolding may overwhelm the proteostasis networks. An interesting question, therefore, is how much misfolding is too much misfolding. We must further consider whether all misfolded and damaged proteins are recognized, refolded or cleared with equal efficiency, or, alternatively, whether certain proteins, such as those implicated in neurodegenerative diseases, are particularly challenging for the quality control machineries.

Specificity in proteostasis networks

Our understanding of the underlying mechanisms responsible for the failure of protein folding homeostasis in conformational disease partly depends on the knowledge of the cell- and tissue-specific composition, regulation and limitations of proteostasis networks. While there are clear indications of cell-type specific expression of some molecular chaperones and components of degradation machinery [54], a comprehensive definition of tissue- and cell-specific expression patterns during organismal development and aging is lacking. Beyond expression, the substrate specificities of different chaperones, even within the same family, their co-chaperone interactions, and the regulation of their activity within the cell are also not well understood. Defining specific chaperone requirements for the folding, maturation and maintenance of the native state for different disease-associated proteins should begin to address these gaps. For example, the recognition and retention of misfolded forms of mutant CFTR specifically depends on a HSP90 co-chaperone AHA1, and downregulation of AHA1 improves CFTR trafficking [55]. This sets the important paradigm that differences in expression or regulation of AHA1 itself, other components of the HSP90 complex, or even other substrates of AHA1, could modulate the fate of CFTR. Co-chaperones, and chaperones with a more restricted expression pattern or substrate repertoire may be particularly important to account for specificity. For example, HSP70 chaperones have been demonstrated in many studies to be important in the regulation of folding, misfolding and aggregation of both normal cellular proteins and of disease-associated proteins. However, apart from compartment-specific HSP70s, we have a rather limited understanding of the specific contributions of each of the ~12 members of HSP70 family to this process, and of their specificity toward protein substrates. A study of J domain-containing proteins in yeast, undertaken for the purpose of molecularly dissecting the HSP70 – J protein network [56], revealed that while many J proteins performed “generalized” functions, a subset of them had non-redundant functions, perhaps by targeting HSP70s to specific substrates [56]. In agreement with this, dHJD1, but not dHJD2, synergized with HSP70 in suppression of polyglutamine toxicity in *Drosophila* [57].

Chaperones regulate both folding and aggregation

It is currently thought that the toxicity of disease-associated aggregation-prone proteins *in vivo* reflects a combination of their intrinsic aggregation propensity and their interaction with individual components of the proteostasis network, such as molecular chaperones. It is well documented that molecular chaperones can influence both the folding trajectories and aggregation pathways of their client proteins [57,58]. A mechanistic insight into this process is provided by a study of the folding and aggregation of the variable domain of immunoglobulin light chain (V_L), mutations in which cause light chain amyloidosis [59]. The HSP70 chaperone, BiP, is necessary for the efficient folding of the V_L *in vivo* [60], and also regulates its amyloid aggregation *in vitro*. Strikingly, one of the two dominant BiP-binding sites on the V_L was directly involved in the formation of amyloid fibrils, as the synthetic peptide containing this site specifically inhibited amyloidogenesis by the V_L [61]. Thus, this chaperone's normal function in promoting efficient folding of its substrate in the cell can also serve to prevent amyloid formation by the mutant forms of this protein in disease, with the same binding site potentially mediating both functions. Another study that echoes this conclusion showed that the anti-oligomer antibody, A11, recognizes a subset of molecular chaperones, and that A11 binding to these chaperones can interfere with the suppression of aggregation or the refolding of substrates [62]. An increased competition for such chaperone from other substrates, particularly if uncompensated for by the stress response, may thus favor the shift toward amyloidogenesis and the formation of toxic species by the disease-associated protein.

Cellular implications of competition for folding resources

The apparent lack of robustness of the proteostasis networks to chronic misfolding is unexpected and has significant implications for late-onset conformational diseases. The aging of an organism is accompanied by an increase in the accumulation of damaged proteins and a decrease in fidelity of biosynthetic processes. If compensatory adaptive responses fail, accumulated misfolded proteins could deplete essential components of the proteostasis machinery [48,51,63]. With as much as 70% of rare missense alleles in human population predicted to be mildly deleterious [64], and approximately half of the genetic changes in inherited disease (in OMIM and HGMD databases) being due to nonsynonymous substitutions [65], such compromise should manifest in a gradual increase in cellular dysfunction and onset of disease. In this scenario, the specific complement of mutations and polymorphisms in expressed genes, in addition to the strength and composition of the proteostasis networks, will establish a dynamic threshold for the onset of dysfunction, both in a cell-specific, and an individual organism-specific manner.

Endogenous metastable proteins suffer when disease proteins misfold

Recent studies in *C. elegans* have shown that expression of expanded polyQ proteins leads to the global disruption of cellular protein folding [66]. This global effect manifests itself in destabilization or misfolding of other expressed metastable proteins, modeled by endogenous temperature-sensitive (ts) folding mutants, with a feedback to polyQ aggregation (Figure 1a–c). As there was no detectable co-aggregation, this mutual destabilization was likely mediated by the competition for folding resources, necessary for maintaining metastable proteins in their folded and functional conformations [66]. In the absence of polyQs, these ts mutants are responsive to the modulation of the folding environment by aging signaling pathways and HSF1 at permissive temperatures, and also show delayed onset of ts phenotypes (Figure 1d,e) [67]. This delayed onset of dysfunction appears to correlate to the onset of misfolding or the mislocalization of the corresponding metastable protein. Likewise, the phenotypic exposure of ts phenotypes in polyQ-containing strains also stemmed from misfolding and aggregation of the ts proteins. Thus, at least in this model, mild folding variants in the genetic background appear to function as a ‘trigger’ for the expression of the toxicity of the aggregation-prone protein, while also channeling the toxicity to tissue-specific phenotypes. Similar destabilization of susceptible proteins, that are either naturally highly dependent on molecular chaperones for their stability and activity, or encoded by destabilizing polymorphisms, could contribute significantly to conformational disease.

The failure to support metastable proteins in their functional state appears to be a common consequence of expression of disease-associated aggregation-prone proteins. It was also observed in a model of SOD1 misfolding, where expression of three different SOD1 mutant proteins – G85R, G93A and a truncated 127X – in strains harboring ts mutations in unrelated genes also resulted in exposure of ts phenotypes at permissive conditions [68]. These mutants of SOD1 exhibit different structural properties and folding trajectories, from G93A that partitions between the native state, characterized by dismutase activity, and tightly aggregated, SDS-sensitive material, to 127X, present only in an unstructured aggregated state, probably due to the large truncation [68]. Considering the likely structured, SDS-resistant aggregation of polyQ expanded proteins, these findings support the view that intracellular toxicity does not need to depend on specific oligomeric structures or folding conformers. In agreement with this view, many of the modifiers of toxicity of polyQ-expanded ataxin-3 in *Drosophila* also rescue the generic toxicity of protein misfolding due to the reduced function of HSP70 [29], and many of the modifiers of polyQ in *C. elegans* also regulate the protection from osmotic-stress-induced protein damage [26,28].

What is the nature of proteins and pathways affected by aggregation-prone disease-associated proteins?

The buffering of ts phenotypes by proteostasis networks is reminiscent of the ability of molecular chaperone HSP90 to buffer phenotypic variation due to cryptic mutations [69]. These cryptic mutations were proposed to be exposed only under (proteotoxic) stress conditions, when the functional availability of HSP90 is limited. Indeed, the phenotypes exposed by the limitation of HSP90 in *Drosophila* correlated to specific genetic backgrounds, and were also affected by temperature. A study in zebra fish showed that developmental phenotypes commonly observed upon HSP90 limitation reflected underlying polymorphisms, whose frequency was strain-specific, while phenotypes that were rarely seen were unique to specific mutant carrier strains [70]. The authors suggested that a similar buffering of underlying polymorphisms may explain an incomplete penetrance observed in human disease. Given that HSP90 is thought to recognize near-native or metastable proteins, these studies imply that, even in the genetically restricted context of laboratory strains of model organisms, there is a surprising amount of normally buffered protein variants that are capable of allowing exposure of phenotypic traits.

Genetic mutations and polymorphisms, genomic instability, mistranslation, or incorporation of amino-acid analogues (such as certain antibiotics or plant metabolites), all have the potential to affect folding pathways and the stability of the native state [52,53,59,71,72]. Coding polymorphisms are not rare and are estimated to occur at an average of two per coding sequence [73], while misincorporation during translation might cause up to 18% of expressed proteins to contain an amino acid substitution [74]. Recent work on the evolution of protein sequences suggests that selection against the toxicity of misfolding due to mistranslation may represent an important evolutionary pressure, when considering highly expressed proteins [74]. From a physiological perspective, this may indicate that the flux of misfolded or destabilized proteins in a cell bears a significant fitness cost. This cost is not only due to the loss-of-function of the misfolded proteins, but also due to the toxic effects of induced aggregation, the consequences of inappropriate intermolecular interactions [9,24,59], and the abnormal engagement of molecular chaperones and degradative machineries. Thus, the cell-type specific complement of expressed protein polymorphisms in functional pathways and complexes could contribute to both the threshold for the onset of proteotoxicity and the ensuing phenotypes in disease.

A model for the cell-specific toxicity in neurodegenerative diseases

We propose a model where the disease-associated aggregation-prone protein is buffered, at least initially, by the cellular folding environment (Figure 2b). Misfolding and aggregation of the disease-associated protein may then reflect the decreased availability of or increased competition for certain limiting components of the proteostasis networks. Such limiting components are likely to be specific to the particular disease-related protein (*i.e.* polyQ *vs.* SOD1), and may or may not be cell-type specific. Aging-related changes in proteostasis networks and stress responses may alone be sufficient to initiate the cascade of misfolding and aggregation of the disease-associated protein. The initiation of the misfolding and aggregation cascade could be accompanied by the general toxicity due to the presence of misfolded species and aggregation intermediates (oligomers), and the altered or inappropriate interactions of these species with other cellular components, in a manner dependent on the identity of the disease-associated protein. On the other hand, if other proteins in the cell (target proteins) compete for the same limiting component(s), the misfolding and aggregation of the disease-associated protein is greatly favored, even before the general compromise of the folding environment. This competition could be either due to the intrinsic instability and chaperone dependence of the target protein, its low translational robustness, or due to destabilizing polymorphisms. The mutual destabilization of the

disease-associated protein and the target protein will additionally contribute to disease as the function of the cellular pathways and protein complexes containing the target protein become compromised, thus imparting the cell- and tissue-specific characteristics to the disease phenotypes (Figure 2d).

Many neurodegenerative and other conformational diseases exist in both familial and sporadic forms. The variants share clinical manifestations but appear to have different underlying causes of disease. The familial variants are associated with mutations that encode aggregation-prone proteins, while the factors contributing to the sporadic disease are largely unknown. Our model suggests that sporadic disease may reflect the dysfunction of the same sensitized pathways and protein complexes that are targeted by the expression of the disease-associated proteins in familial disease (Figure 2c). Several different mechanisms may contribute to this dysfunction. First, there could be a general compromise of the proteostasis network. Such a general effect could be caused by aging-related changes, or by environmental factors, such as proteotoxic stresses, inflammatory and febrile conditions, *etc.* A general effect could also be caused by genetic mutations that impair the proteostasis network by altering its capacity or robustness. Second, mutations in factors that are not usually associated with familial forms of disease could lead to an increased competition for the limiting components of the proteostasis network. In this case, either a single substrate destabilized by sporadic mutation, or several mildly destabilized substrates (for example due to coding polymorphisms or mistranslation), could mimic the competition present in the familial disease. Finally, the target pathways or complexes themselves may be affected directly, by damage or by mutations/polymorphisms in their different protein components, thereby causing disease.

Identification of TDP43 [75] as a protein commonly affected both in familial and sporadic cases of FTL-D-U [76] and ALS [77] could provide an example for this paradigm: the dysfunction of TDP43 itself, or of the cellular pathway in which it functions, brought about by different mechanisms, could underlie the cellular dysfunction in these diseases. Interestingly, TDP43 pathology is not present in the familial ALS cases associated with SOD1 mutations, potentially indicating that ALS with and without SOD1 mutations are distinct disorders. Alternatively, the cellular pathway in which TDP43 functions may itself be affected by the expression of mutant SOD1. This underscores the importance of identifying the target pathway that is directly responsible for the cellular toxicity in conformational diseases for both diagnosis and disease intervention.

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Figure 1.

a–b. Expression of Q40-YFP (Q40, green) in nematodes carrying a destabilizing mutation in paramyosin (par(ts), red) causes mislocalization (**a**) and misfolding (**b**) of paramyosin. **a**, immunostaining and epifluorescence; **b**, limited proteolysis. **c.** Q40-YFP itself displays early onset of aggregation when expressed in a ts background. **a–c** are adapted from Gidalevitz *et al. Science* 2006; 311(5766):1471–4. **d.** Mislocalized par(ts) (green) in an otherwise WT background is seen as early as day 3 of adulthood, and is similar in appearance to the par(ts) at the restrictive temperature (**a**). Actin filaments counterstained in red. **e.** Quantification of the number of cells with disrupted myofilaments in par(ts) or WT worms. **d–e** are adapted from Ben-Zvi *et al.*, *Proc Natl Acad Sci U S A.* 2009;106(35):14914–9.

Figure 2.

a–b. Proteostasis networks buffer metastable and misfolded species, ensuring normal cellular function. **c–d.** Failure of proteostasis networks to buffer misfolding results in onset of dysfunction. Accumulated protein damage, proteotoxic stresses and aging all contribute to the increase in misfolding and thus to the decline in the proteostasis network capacity. Either a single dominant mutation (**d**), or a cumulative effect of milder destabilizing mutations and polymorphisms (**c**) may lead to the cell-specific dysfunction of sensitized pathways and protein complexes, by competing for a shared limiting component(s) of the proteostasis network.