Polyglutamine misfolding in yeast

Toxic and protective aggregation

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Protein misfolding is associated with many human diseases, including neurodegenerative diseases, such as Alzheimer disease, Parkinson disease and Huntington disease. Protein misfolding often results in the formation of intracellular or extracellular inclusions or aggregates. Even though deciphering the role of these aggregates has been the object of intense research activity, their role in protein misfolding diseases is unclear. Here, I discuss the implications of studies on polyglutamine aggregation and toxicity in yeast and other model organisms. These studies provide an excellent experimental and conceptual paradigm that contributes to understanding the differences between toxic and protective trajectories of protein misfolding. Future studies like the ones discussed here have the potential to transform basic concepts of protein misfolding in human diseases and may thus help to identify new therapeutic strategies for their treatment.

Misfolded Proteins, Inclusions and Amyloids in Disease and Normal Biology

Many human diseases, particularly a great number of neurodegenerative diseases, are tightly connected to protein misfolding.¹ In these diseases, specific proteins convert from their normal functional conformations into abnormal, disease-related conformations.² Frequently, protein misfolding results in the formation of large cellular protein deposits, called inclusions.³

Inclusions are often composed of proteins in highly stable ordered conformations, called amyloids.⁴ Amyloids are structurally characterized by a cross-beta core structure with individual beta-strands aligning orthogonal to the fiber axis.⁵ In addition, amyloids have the unique capacity to trigger the conversion of soluble proteins into the same amyloid structure. These properties distinguish amyloids from other, less ordered protein aggregates.

Proteins with diverse amino acid sequences can form amyloid-like conformations.⁶ Prominent examples for amyloid-forming disease-related proteins include: A β and tau in Alzheimer disease, alpha-synuclein in Parkinson disease, the prion protein (PrP) in prion diseases, such Creutzfeld-Jakob disease or mad cow disease, and polyQ-expansion proteins in polyQ-expansion diseases, such

as Huntington disease or the Spinocerebellar Ataxias.³ Extra- or intra-cellular amyloid inclusions are established as pathological hallmarks of all of these diseases. Yet it is unclear what role the amyloid inclusions play in mediating the toxicity associated with protein misfolding.⁷

Many earlier pathological observations and experiments have indicated that amyloid-like inclusions are tightly linked to disease or may even present the one distinct protein species that causes toxicity and triggers disease:⁸ (1) as observed by post mortem cytohistochemistry, the regions of the brain that are most drastically affected by a neurodegenerative disease very often have the highest load of amyloid-like aggregates; (2) overexpression of the disease-associated misfolded proteins results in massive amyloid-like aggregation in cell and animal models; (3) familial variants of neurodegenerative disease show a particularly high load of amyloid-like aggregates; (4) the mutant alleles of misfolded proteins that cause these familial variants of neurodegenerative diseases also cause massive amyloid-like aggregation when expressed in cell and animal models; (5) in vitro, the disease-associated, misfolded proteins and the mutant alleles of these proteins that cause the familial variants of neurodegenerative diseases have a strong propensity to undergo amyloid formation.

All of these features seem to indicate that inclusions or aggregates are key disease-causing protein species in protein misfolding diseases. Accordingly, eradicating amyloid-like inclusions emerged as a dominant therapeutic target for the treatment of protein misfolding diseases.^{9,10} In fact, many small molecules and protein fragments have been identified that can prevent the formation of amyloids or even solubilize already formed amyloids. Many studies have documented that such anti-amyloid reagents can have positive effects on protein misfolding diseases, most notably in models for neurodegenerative diseases.¹¹

Recent findings have challenged the view that amyloid-like protein conformations are the major toxic species in disease and suggest that more soluble protein species are the main culprit.^{12,13} Pathological studies indicate a poor correlation between amyloid load and dementia or other symptoms of neurodegeneration.⁷ In fact, even symptom-free individuals can present strong accumulation of amyloid deposits. In contrast, the load of oligomeric protein species correlates much better with symptoms of neurodegeneration in patients and this is also recapitulated in many cell and animal models.¹³ Such oligomeric protein assemblies can be highly toxic to cells and thus may present the disease-causing protein species.¹⁴

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Because increasing numbers of studies provide evidence for the toxic role of the more soluble oligomeric protein conformers, the role of the larger insoluble amyloid-like inclusions in disease has been redefined: amyloids are regarded as epiphenomenal, i.e., without any major role in disease; alternatively, amyloids have been suggested to be protective, e.g., by preventing or reducing the formation of the more toxic oligomeric protein species or by reducing the surface area of toxic protein assemblies and thus reducing toxic interactions.⁷

As a consequence, eradicating amyloids may not be a valuable therapeutic target for the treatment of protein misfolding diseases. On the contrary, dissolving amyloids might result in increased formation of highly toxic oligomeric protein conformations and might ultimately aggravate the pathological state of patients.

On a different note, recent studies indicate that certain amyloid-like proteins are not toxic at all. On the contrary, work in bacteria, yeast, slugs and mammalian cells demonstrate that amyloid-like proteins can perform normal physiological functions.¹⁵ Examples for these functional amyloids include: the protein curli, which forms functional amyloid-like proteins important for formation of bacterial biofilms;¹⁶ the protein CPEB, whose amyloid-like conformers act during long-term potentiation in the slug *Aplysia*;¹⁷ the protein Pmel17, which in its amyloid conformation aids the synthesis of melanin in mammalian melanosomes;¹⁸ peptide and protein hormones in amyloid conformations in the pituitary secretory granules of the mammalian endocrine system;¹⁹ and the yeast prions,¹⁵ which can switch between a soluble and an amyloid conformation to regulate translation termination (as shown for the yeast prion [PSI⁺]), or regulate nitrogen metabolism (as shown for the yeast prion [URE3⁺]). These amyloid-like yeast prions may even serve as capacitors for the adaptation to changing environments and may thus facilitate the evolution of new phenotypes.^{20,21}

These phenomena indicate that amyloids may not represent exclusively toxic protein conformations but that amyloids play important roles in regular biology. Therefore, generally targeting amyloids and eradicating them to treat protein misfolding diseases may result in the elimination of benign and functional amyloids and thus interfere with important physiological processes.

In summary, the role of amyloids in the pathogenesis of protein misfolding diseases is still enigmatic. This lack of a defined role of amyloid-like protein conformation in disease and the burgeoning evidence for benign and functional amyloids suggest that indiscriminately dissolving amyloids may not present ideal therapeutic strategy.

PolyQ Diseases

An intriguing vantage point from which to study the problem of protein misfolding, including the role of amyloids and the role of oligomeric protein species in human diseases, are the polyglutamine (polyQ) expansion diseases.²²⁻²⁴ Nine different diseases are caused by mutations that result in the expansions of polyQ regions in nine different proteins. In all of these diseases, the polyQ-expansion leads to the misfolding of the polyQ-expanded

protein, its aggregation into large cellular inclusions, cytotoxicity and finally the dysfunction and the demise of specific neurons. Also, in all of these diseases, larger polyQ-expansion results in a more pronounced misfolding of the polyQ-expansion protein, an earlier onset of disease symptoms, and a more rapid and severe course of the diseases.²³

Disease-related polyQ-expansions were found in nine different proteins with different cellular functions (if identified) and localizations:²²⁻²⁴ in the protein huntingtin, which causes Huntington disease; in the proteins Sca1, Sca2, Sca3, Sca6, Sca7 and Sca17, which cause different types of Spinocerebellar Ataxias; in the androgen receptor protein, which causes Kennedy Disease (also known as spinobulbar muscular atrophy, SBMA); and in the protein atrophin-1, which causes dentatorubro-pallidoluysian atrophy (DRPLA).

The different polyQ-diseases cause perturbations in diverse cellular pathways, which affect specific neurons in specific regions of the brain and thus result in distinct pathological features. It is plausible that the different proteins which harbor the polyQ-expansions and the individual expression patterns of each of the nine polyQ-expansion proteins elicit distinct pathological features. Yet in any case, polyQ-expansions represent protein regions that—irrespective of their specific neuronal or protein context—are toxic to cells and govern the formation of misfolded proteins, oligomeric protein species and large amyloid-like inclusions.

Yeast Models of Protein Misfolding

The yeast *Saccharomyces cerevisiae* serves as a genetically and biochemically tractable experimental platform to study protein misfolding and its ensuing toxicity.²⁵⁻²⁸ Yeast models for studying diverse misfolded proteins have been established. These models express: alpha-synuclein, which is associated with Parkinson disease;²⁹ TDP-43 (TAR DNA-binding protein 43),³⁰ which is associated with Amyotrophic Lateral Sclerosis (ALS) and frontotemporal lobar dementia (FTLD); polyalanine-expanded proteins,³¹ which are associated with nine different human diseases (oculopharyngeal muscular dystrophy (OPMD), syndactyly type II, cleidocranial dysplasia, holoprosencephaly, hand-foot-genital syndrome, blepharophimosis ptosis and epicanthus inversus, X-linked mental retardation, X-linked infantile spasm syndrome and congenital central hypoventilation syndrome); FUS (fused in Sarcoma),³²⁻³⁴ which is associated with ALS; and polyQ-expansion proteins,³⁵⁻³⁹ which are associated with polyQ-expansion diseases (see above).

Importantly, these yeast models recapitulate key molecular and cellular aspects of the particular misfolded proteins and their corresponding diseases. Consequently, yeast models have opened new avenues to identify molecular pathways and modifiers underpinning protein misfolding and its ensuing toxicity.

Yeast PolyQ Models

The first yeast model expressing a polyQ-expansion protein was introduced by Krobisch and Lindquist.³⁷ Short fragments of the protein huntingtin (Htt) with different lengths of their

polyQ-expansions were expressed in yeast. Notably, these short fragments of polyQ-expanded Htt have been expressed in cultured mammalian cells and mice before and these cell and mouse models recapitulate central aspects of HD, including polyQ length-dependent protein aggregation and toxicity.

The aggregation of polyQ-expanded Htt in yeast was monitored by using fusions of fluorescent proteins to the carboxy-terminus of Htt fragments. While the expression of a Htt fragment with a short, non-disease-related polyQ-lengths (25Q) produced soluble protein, Htt fragments with expanded polyQ regions in the disease-related range (47Qs, 72Qs and 103Qs) produced insoluble inclusions. Htt fragments with increasing polyQ-expansions produced increasing amounts of insoluble protein. Importantly, however, the polyQ-expansion proteins were not toxic to yeast cells, as yeast cells expressing even very high concentrations of these proteins proliferated normally and showed no obvious sign of cellular toxicity. Similarly, Muchowski et al. established a yeast polyQ model that recapitulated polyQ length-dependent aggregation, yet also no polyQ length-dependent toxicity.³⁹

Perplexingly, Meriin et al. published a study demonstrating both polyQ length-dependent aggregation and toxicity in their yeast model.³⁸ Our ensuing systematic comparison of the different yeast polyQ models established the prerequisites for polyQ toxicity in yeast (see below for a detailed analysis).^{35,36} In brief, we found that polyQ toxicity in yeast is strongly modulated by the amino acid sequences that flank the polyQ-expanded region at its amino- and its carboxy-terminus within the Htt fragment. Furthermore, the expression and conformation of other proteins in yeast, most importantly the expression and conformation of the protein Rnq1, determines whether polyQ-expanded Htt fragments were toxic or benign.^{35,38}

The toxic polyQ models have served as a platform to decipher the molecular mechanisms underpinning polyQ toxicity. For instance, Giorgini et al. identified the kynurenine pathway, a pathway involved in degradation of tryptophan, as central to polyQ toxicity in a yeast screen for modifiers of polyQ toxicity.⁴⁰ Also, Meriin et al. explored the role of endocytosis in polyQ toxicity in yeast.⁴¹ Finally, our yeast studies documented that the impairment of ER (endoplasmic reticulum) associated protein degradation (ERAD) and the ensuing ER stress are central to polyQ toxicity.⁴² Notably, the non-toxic polyQ-expanded Htt fragments do not interfere with any of these cellular pathways.

PolyQ Toxicity in Yeast: The Prion [RNQ⁺]

Gokhale et al. Meriin et al. and Osherovich et al. found that polyQ-expanded fragments of Htt or polyQ-expanded MJD (Sca3) are only toxic in yeast cells that harbor the protein Rnq1p in its prion conformation, [RNQ⁺].⁴³⁻⁴⁵ By contrast, in [*rnq*⁻] cells or cells bearing a deletion of the gene encoding Rnq1 (Δ rnl), polyQ-expanded htt fragments do not form intracellular inclusions and do not produce any toxicity.

These studies generally suggest that other misfolded or aggregated proteins, such as Rnq1 in its prion conformation [RNQ⁺], seed or enhance the aggregation of polyQ-expansion proteins. In agreement with this notion, our experiments showed that

the co-expression of other polyQ proteins together with polyQ-expanded Htt fragments increases polyQ aggregation and toxicity in yeast.³⁵ Yet [RNQ⁺] is by far most effective in inducing polyQ aggregation and toxicity. [RNQ⁺] is also required to trigger the aggregation of non-toxic polyQ-expanded Htt fragments, i.e., [RNQ⁺] seems to be required, yet is not sufficient, to produce polyQ toxicity in yeast.

Overall, these results indicate that in protein misfolding diseases, the aggregation and toxicity of any aggregating protein may be strongly modulated by the presence of other misfolded or aggregated proteins. Importantly, these studies demonstrate that triggering the aggregation of polyQ-expansion proteins is necessary to convey polyQ toxicity but aggregation is not sufficient to trigger polyQ toxicity. It is important to emphasize, however, that this requirement for aggregation does not indicate that the aggregates per se are toxic. These results only indicate that processes that result in aggregate formation may be required to produce toxic protein species. Clearly, further experiments are required to validate this general concept in the context of polyQ-expansion proteins and also with other disease-related, aggregation-prone proteins.

PolyQ Toxicity in Yeast: Flanking Regions Determine PolyQ Toxicity

Our detailed analysis of different Htt fragments in yeast revealed that the amino acid sequences that flank the polyQ-expansions are critical determinants of polyQ toxicity in yeast.³⁶ We compared the aggregation and toxicity of seven different Htt fragments. One polyQ Htt variant, variant II, produces polyQ toxicity in a polyQ length-dependent manner that is characteristic for all nine polyQ diseases: polyQ Htt proteins of variant II with 46Qs caused mild toxicity, whereas polyQ Htt of variant II with 72Qs and 103Qs produce high levels of toxicity. Htt variant II with only 25Qs is not toxic.

The toxic polyQ Htt constructs contain an amino-terminal Flag-tag and do not contain the proline-rich region (P₁₁QLP QPP PQA QPL LLP QPQP₁₀) that is found adjacent to the carboxy-terminus of the polyQ region within the short, amino-terminal fragment of human Htt. Here, I focus on the impact of the proline-rich region on polyQ toxicity. The fascinating impact on polyQ Htt aggregation and toxicity of amino-terminal modifications and the 17 amino acids that flank the polyQ region in Htt at its amino-terminus (N17) were recently reviewed in references 46–48.

PolyQ Htt constructs that contain the proline-rich region never produce toxicity in yeast, regardless of the expression levels or experimental conditions.^{36,38,49} This indicates that the proline-rich region protects from polyQ toxicity in cis, i.e., when adjacent to the polyQ region. Amazingly, we and later Wang et al. found that the proline-rich region can inhibit polyQ toxicity when expressed in trans, i.e., when the toxic polyQ-expanded htt is co-expressed with an Htt fragment containing 25Qs and the proline-rich region.^{36,50}

Fluorescence microscopy using polyQ Htt fragments carboxy-terminally fused to fluorescent proteins showed that the toxic

variant II protein formed amorphous aggregates dispersed over the yeast cytosol.^{36,38,50} By contrast, non-toxic polyQ Htt variants that contain the proline-rich region formed one single, tight, perinuclear aggregate per cell.^{36,50,51} Biophysical assays showed that both toxic and non-toxic Htt variants produce SDS-resistant aggregated protein species. Notably, the toxic variant II proteins seem somewhat more soluble than their corresponding non-toxic variants.

To our knowledge, this is the first example of a short amino acid sequence that can convert a toxic aggregated protein into a benign protein aggregate and vice versa in the exact same cell type (clonal, isogenic yeast cells). The short proline-rich region also dramatically altered the cellular polyQ aggregation pattern. In a series of nifty genetic and proteomic experiments, Wang et al. took advantage of this unique experimental vantage point and explored the role of the protective proline-rich region in polyQ-expanded Htt.⁵⁰ They conclude that the proline-rich region triggers the formation of the yeast version of non-toxic aggresomes.

In metazoan cells, aggresomes are defined as cellular structures localized to centrosomes that harbor misfolded proteins.^{52,53} The formation of aggresomes depends on a functional microtubule network. Aggresomes accumulate polyQ-ubiquitinated proteins and proteins involved in cellular proteostasis, such as molecular chaperones and proteins of the ubiquitin-proteasome system (UPS). The formation of aggresomes appears to be a protective mechanism that allows cells to sequester misfolded proteins (e.g., upon proteasome inhibition) and either facilitate their refolding or their degradation by the UPS or autophagy.

In yeast, the proline-rich region in polyQ-expanded Htt, elicits the formation of a benign, microtubule-dependent, aggresome-like structure. The proteins Cdc48 (the yeast version of mammalian VCP or p97), Npl4 and Ufd1 appear to be central to the formation of this aggresome-like structure.⁵⁰ The trimeric complex of Cdc48, Npl4 and Ufd1 is involved in the recognition of poly-ubiquitinated proteins and their delivery to the proteasome during ER associated protein degradation (ERAD).⁵⁴ Understanding the mechanistic role of this ERAD complex during aggresome formation requires further investigation.

It is noteworthy that studies using intracellular antibodies (intrabodies) that specifically recognize the proline-rich region of Htt also imply a crucial role of the proline-rich region in polyQ Htt aggregation and toxicity. Binding of these intrabodies to the proline-rich regions within polyQ-expanded Htt alters aggregation and reduces toxicity.^{55,56}

Furthermore, biophysical studies have explored the impact of the proline-rich region on polyQ aggregation in vitro.⁵⁷ A polyproline-region carboxy-terminally adjacent to a polyQ region decreases the rate of polyQ amyloid formation and decreases the stability of amyloids as compared to pure polyQ proteins. The effects of the polyproline-region within the polyQ protein may be explained by competing secondary structures of each of these domains: the polyQ region favors a beta-sheet structure, whereas the poly-proline region favors proline-type-two helix (PPII helix), which antagonizes the formation of a beta-sheet-rich amyloid structure.

The results summarized above clearly indicate a strong impact of the proline-rich region on polyQ Htt aggregation and toxicity.

The combination of two major forces seems to explain this effect: the impact of the proline-rich region on polyQ conformations—as documented by the biophysical studies—and the cellular interactions of the proline-rich region which leads to the formation of a protective polyQ aggregate (aggresome)—as documented by the results obtained from yeast models. Future mechanistic studies will reveal how precisely these two different effects of the proline-rich region modulate polyQ Htt aggregation and toxicity in cells.

Other disease related polyQ-expansion proteins (atrophin-1, ataxin1, ataxin2 and ataxin7) also contain proline-rich regions adjacent to their polyQ regions, albeit with lower proline contents than the region within Htt.⁵⁷ It will be informative to determine whether the proline-rich regions in these proteins also have the potential to modulate polyQ aggregation and polyQ toxicity. One could even speculate that proline-rich regions evolved adjacent to polyQ regions to keep the potentially baneful effects of the polyQ regions under control.

Worm, Fly and Tissue Culture Models of PolyQ Aggregation and Toxicity

Several groups have established the nematode *Caenorhabditis elegans* as a powerful model system to study protein aggregation and toxicity, including the aggregation and toxicity of polyQ expansion proteins.^{58,59} The expression of polyQ-expansion proteins in diverse tissues (e.g., muscle wall cells and neurons) of *C. elegans* results in polyQ aggregation and toxicity.⁶⁰ The worm model has been particularly informative in exploring the interplay between aging and polyQ aggregation and toxicity as well as the interplay between the cellular proteostasis network and polyQ aggregation and toxicity.^{61,62}

In the context of this review, a study by Faber et al. using a worm polyQ model is of particular interest.⁶³ In a genetic screen aimed to identify modifiers of polyQ toxicity, the authors found that the overexpression of the glutamine- and proline-rich protein, PQE-1, efficiently suppressed polyQ toxicity. They also found that the loss of PQE-1 function exacerbated polyQ toxicity. In light of the yeast results discussed above, it is tempting to speculate that PQE-1 functions as an aggresome forming element acting in trans. Like the proline-rich region, it transforms misfolded toxic polyQ-expansion protein into benign aggresomes. Further studies are required to determine the mechanism by which PQE-1 modulates polyQ toxicity.

The fruit fly *Drosophila melanogaster* also serves as a powerful model to study protein misfolding, including polyQ aggregation and toxicity.⁶⁴ Disease-related polyQ expansion proteins expressed as full-length versions or fragments in flies recapitulate many key features of the corresponding polyQ-expansion disease, including HD, SCA1, SCA3 and Kennedy Disease.^{65–68} It will be interesting to compare these different polyQ-expansion proteins to each other and their individual capacities to produce toxic misfolded protein species or more protective, aggresome like structures.

Countless cultured mammalian cell-types have been used to study cell biological aspect of polyQ aggregation and toxicity.

Notably, all of these mammalian cell culture models recapitulate polyQ length-dependent aggregation, regardless of the precise amino acids flanking the polyQ region. Yet polyQ length-dependent toxicity varies substantially from one model to the others. For instance, the PC12 cell line derived from a rat pheochromocytoma (a tumor) expressing a polyQ-expanded Htt fragment produces high degrees of toxicity in one model⁶⁹ yet is not toxic in another.^{69,70} The reasons for this fascinating discrepancy are unclear at this point and probably difficult to address in a cellular model that is genetically as unstable as a tumor cell line. Perhaps proteomic comparisons of the interacting proteins in the two different PC12 polyQ models can deliver some insights into the molecular mechanism underpinning this profound difference in polyQ toxicity.

Work in mammalian tissue culture models has described aggresome formation of polyQ-expansion proteins.⁷¹ It has also been indicated that these aggresome-like polyQ structures present a protective mechanism even though it is not clear what drives this protective aggresome formation.⁷² In agreement with this notion, an elegant microscopic study by Arrasate et al. strongly correlated the absence of big (perhaps aggresome-like) cellular inclusions with increased polyQ toxicity.⁷³ By contrast, cells with mostly diffuse polyQ staining showed a high degree of polyQ toxicity.

A study by Bodner et al. identified a small molecule that reduced defects in protein degradation caused by the expression of a polyQ-expanded Htt fragment in PC12 cells.⁷⁴ Notably, this small molecule enhanced polyQ aggregation and the aggregation of other misfolded proteins.

In summary, the results derived from experiments in worm, fly and cell-culture models indicate a complex yet enticing nexus between polyQ aggregation and the formation of aggresomes. It will be fascinating to explore the power of each of these experimental systems, in combination with work in yeast models and in vitro biochemistry, to address the many mysteries surrounding polyQ-expansion proteins, their aggregation and their toxicity.

Outlook

As exemplified by the yeast studies discussed above, it will be crucial to systematically analyze polyQ-expansion proteins with different flanking sequences in the exact same (isogenic) cell types or animals and at the exact same expression conditions to further

determine what triggers polyQ-toxicity and what prevents it. A direct comparison of toxic and benign polyQ proteins may reveal which structural conformers cause polyQ toxicity, possibly by inhibiting the formation of protective aggregates. Also, this comparison might unveil specific polyQ conformers that facilitate the formation of benign aggregates and aggresomes.

Furthermore, it will be critical to determine how cellular proteostasis contributes to the formation of benign, aggresome-like polyQ aggregates: which components of cellular proteostasis are required? Does cellular proteostasis prevent the formation of toxic misfolded polyQ species or does it remodel toxic polyQ protein species into benign aggresomes? Or can it do both? Dissecting the molecular mechanism by which proline-rich regions and the protein complex consisting of p97 (Cdc48), Ufd1 and Npl4 contribute to the formation of protective polyQ aggresomes could pave the way for discoveries that will elucidate the biochemical and cellular trajectories of toxic protein misfolding and the protective aggresome formation. Such studies might also clarify how cellular proteostasis may fail to provide these protective function, e.g., in aging cells.

The polyQ-expansion proteins offer an ideal experimental platform from which to explore the biochemical, genetic and cellular processes guiding toxic and protective protein aggregation. These studies may well lay the experimental and conceptual foundations to investigate the misfolding of other disease-related misfolded proteins and find key factors that guide their misfolding into either toxic or non-toxic protein species. PolyQ studies may thus aid to decipher the mechanisms underpinning the formation of aggregated proteins that function in normal biology. Finally, a detailed understanding of the principles of benign and toxic protein misfolding could establish the formation of benign protein aggregates, such as aggresomes, as a promising therapeutic target for the treatment of many protein misfolding diseases, including the polyQ-expansion diseases. Possibly, soon we will be screening for genes and small molecules that enhance protein aggregation rather than inhibiting it.

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