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Defining the Limits: Protein Aggregation and Toxicity *In vivo*

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Abstract

The proper folding of proteins to their functional forms is essential to cellular homeostasis. Perhaps not surprisingly, cells have evolved multiple pathways, some overlapping and others complementary, to resolve misfolded proteins when they arise, ranging from refolding through the action of molecular chaperones to elimination through regulated proteolytic mechanisms. These protein quality control pathways are sufficient, under normal conditions, to maintain a functioning proteome, but in response to diverse environmental, genetic, and/or stochastic events, protein misfolding exceeds the corrective capacity of these pathways, leading to the accumulation of aggregates and ultimately toxicity. Particularly devastating examples of these effects include certain neurodegenerative diseases, such as Huntington's Disease, which are associated with the expansion of polyglutamine tracks in proteins. In these cases, protein misfolding and aggregation are clear contributors to pathogenesis, but uncovering the precise mechanistic links between the two events remains an area of active research. Studies in the yeast *Saccharomyces cerevisiae* and other model systems have uncovered previously unanticipated complexity in aggregation pathways, the contributions of protein quality control processes to them, and the cellular perturbations that result from them. Together these studies suggest that aggregate interactions and localization, rather than their size, are the crucial considerations in understanding the molecular basis of toxicity.

Introduction

While the pioneering work of Christian Anfinsen demonstrated that the sequence of amino acids in a polypeptide chain is sufficient to direct its proper folding (Anfinsen, 1967), we now appreciate that the same sequence allows considerable variation in folding trajectory to the native state, including off-pathway alternative states that are often associated with pathogenesis (Jahn and Radford, 2008). These sequence-based challenges to protein folding are also compounded by additional limitations, such as vectorial synthesis, molecular crowding, environmental and metabolic stresses, aging, mutations and synthesis errors, which are specific to the cellular environment (Kim et al., 2013). In the vast majority of

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cases, cellular quality control pathways act to maintain protein homeostasis (proteostasis) through the reactivation or clearance of aberrantly folded proteins, but in other cases, these pathways become overwhelmed leading to the accumulation of misfolded proteins, the disruption of normal cellular activities, and ultimately disease (Balch et al., 2008).

Many “protein misfolding” diseases are associated with a special group of metastable proteins that can access non-native conformations with a propensity to assemble into β -sheet-rich fibers (Chiti and Dobson, 2006). These complexes, known as amyloid, are characterized by detergent resistance, high thermodynamic stability, and the ability to continually incorporate monomers of the same protein, effectively titrating these species from a productive folding pathway to the native state and thereby self-replicating the amyloid state (Jahn and Radford, 2008). Together, the stability and self-replicating nature of amyloid fibers contributes to their persistence by protecting these complexes from complete disassembly by protein quality control pathways *in vivo* (Tuite and Serio, 2010).

The accumulation of amyloid can be toxic to eukaryotes from yeast to man, but naturally occurring amyloid can also be tolerated benignly and can even contribute functionality, including the regulation of sterol biosynthesis (Suzuki et al., 2012), hormone storage (Maji et al., 2009), organelle biogenesis (Fowler et al., 2006, Berson et al., 2003), memory (Si et al., 2010), nutrient sensing (Brown and Lindquist, 2009), transcription (Wickner, 1994, Du et al., 2008, Patel et al., 2009, Rogoza et al., 2010), and translation (Patino et al., 1996, Paushkin et al., 1996). These observations suggest that the amyloid structure and its assembly intermediates *per se* are not toxic, but rather that attributes of the constituent proteins themselves and their interactions with their cellular environments specifically mediate toxicity. Studies in many model systems, but particularly in the yeast *Saccharomyces cerevisiae*, have begun to systematically dissect the impact of both protein-specific and cell-based factors likely to mediate toxicity and of the interactions among these contributors. Here, we synthesize recent work in this area for two classes of proteins: prions, which are transmissible between individuals through either a heritable or infectious route, and polyglutamine (polyQ)-expanded proteins that are non-transmissible but likely to spread among cells within an organism (Aguzzi, 2009, Brundin et al., 2010, Li et al., 2008, Meyer-Luehmann et al., 2006, Ren et al., 2009, Tuite and Serio, 2010). Together, these studies suggest that conditions that create imbalances in aggregation and clearance pathways lead to toxicity by altering aggregate dynamics, localization and resulting interactions (Figure 1).

Sequestration as a Mechanism of Prion Toxicity in Yeast

The yeast *Saccharomyces cerevisiae* is known to propagate ten endogenous prions, (Aigle and Lacroute, 1975, Alberti et al., 2009, Brown and Lindquist, 2009, Cox, 1965, Derkatch et al., 1997, Du et al., 2008, Patel et al., 2009, Rogoza et al., 2010, Wickner, 1994, Halfmann et al., 2012) with another nearly 20 candidate prions awaiting further characterization (Alberti et al., 2009). Of the confirmed prions, $[PSI^+]$ (Cox, 1965), the prion form of the Sup35 protein (Chernoff et al., 1995, Patino et al., 1996, Paushkin et al., 1996, Wickner, 1994), $[PIN^+]/[RNQ^+]$ (Derkatch et al., 1997), the prion form of the Rnq1 protein (Derkatch et al., 2001, Osherovich and Weissman, 2001, Sondheimer and Lindquist, 2000), and $[URE3]$ (Aigle and Lacroute, 1975), the prion form of the Ure2 protein (Masison and

Wickner, 1995, Wickner, 1994), are the most extensively studied. While some self-replicating conformations (variants) of these proteins are toxic, other variants of $[PSI^+]$, $[URE3]$, and $[RNQ^+]$ are not detrimental to yeast under normal laboratory growth conditions (McGlinchey et al., 2011, Halfmann et al., 2010). Nevertheless, these benign isolates can become toxic in the case of $[PSI^+]$ and $[RNQ^+]$ with dose-dependent increases in the levels of the Sup35 or Rnq1 proteins, respectively (Douglas et al., 2008, Chernoff et al., 1993, Chernoff et al., 1992, Dagkesamanskaya and Ter-Avanesyan, 1991, Vishveshwara et al., 2009, Zhou et al., 1999). Importantly, toxicity requires the presence of the $[PSI^+]$ or $[RNQ^+]$ prions, suggesting a transition in the interaction of the underlying Sup35 or Rnq1 amyloid structures, or of their assembly intermediates, with the cellular environment at higher doses (Douglas et al., 2008, Dagkesamanskaya and Ter-Avanesyan, 1991, Vishveshwara et al., 2009, Zhou et al., 1999). Despite their unrelated sequences and targets, parallel mechanisms underlie the toxicity of each protein.

In its non-prion form, Sup35 is a GTPase that functions as the eukaryotic release factor 3 (eRF3) to stimulate both peptidyl-tRNA hydrolysis by, and recycling of, the eukaryotic release factor 1 (eRF1, Sup45) (Stansfield et al., 1995, Zhouravleva et al., 1995, Eyler et al., 2013, Alkalaeva et al., 2006). In its $[PSI^+]$ prion form, up to ~90% of Sup35 assembles into SDS-resistant aggregates of heterogeneous size, and this shift in oligomerization is associated with a translation termination defect (Pezza et al., 2009, Kryndushkin et al., 2003, Patino et al., 1996, Paushkin et al., 1996, Tanaka et al., 2006, Derkatch et al., 1996, Cox, 1965, Liebman and Sherman, 1979). The translation termination functions of Sup35, including its interaction with Sup45, are primarily mediated by the C-terminal domain of the protein (amino acids 254–685), while the N-terminus of the protein (amino acids 1–253) supports prion propagation (Ter-Avanesyan et al., 1994, Ito et al., 1998, Paushkin et al., 1997).

The N-terminal prion-determining domain (PrD) is also required for overexpression-mediated toxicity in a $[PSI^+]$ strain, suggesting that assembly of the protein into amyloid is required for this effect (Derkatch et al., 1996, Ter-Avanesyan et al., 1993, Vishveshwara et al., 2009). Consistent with this idea, overexpression of the functional domain of Sup35, which cannot be incorporated into aggregates in a $[PSI^+]$ strain (Ter-Avanesyan et al., 1994), is sufficient to suppress the toxicity induced by overexpression of the PrD, although it is ineffective in suppressing the toxicity associated with overexpression of full-length Sup35 (Vishveshwara et al., 2009). In this latter case, overexpression of Sup45 is required to suppress toxicity (Vishveshwara et al., 2009, Derkatch et al., 1998, Stansfield et al., 1995, Tank and True, 2009, Gong et al., 2012). Together, these observations suggest that upon overexpression, Sup35 or its PrD sequesters the residual functional pool of Sup35 in a $[PSI^+]$ strain. Because Sup45 retains the ability to interact with aggregated Sup35 in a $[PSI^+]$ strain (Czaplinski et al., 1998, Paushkin et al., 1997, Gong et al., 2012), overexpression of full-length Sup35 also leads to sequestration of Sup45 (Vishveshwara et al., 2009). In either case, toxicity likely results from a reduction in the availability of these factors to perform their normal functions (Chernoff et al., 1992, Valouev et al., 2002).

In $[RNQ^+]$ cells overexpressing Rnq1, toxicity has been linked to a cell cycle arrest in mitosis at the Mad2 spindle checkpoint, resulting from a failure to duplicate the spindle pole

body (Treusch and Lindquist, 2012). These effects were linked to Spc42, a core component of the spindle pole body (Bullitt et al., 1997, Treusch and Lindquist, 2012). Spc42 co-localizes with Rnq1 to cytoplasmic foci distinct from the spindle pole body in a $[RNQ^+]$ but not in a non-prion $[rnq^-]$ strain, and overexpression of Spc42 relieves Rnq1-mediated $[RNQ^+]$ toxicity, implicating sequestration once again as the mechanism of toxicity (Treusch and Lindquist, 2012). Unlike Sup35-mediated $[PSI^+]$ toxicity however, overexpression of the Rnq1 PrD in a $[RNQ^+]$ strain is not toxic (Douglas et al., 2008, Summers et al., 2009b). While these observations suggest a role for the non-prion domain in toxicity, overexpression of this region of the protein does not suppress the toxicity of full-length Rnq1 in a $[RNQ^+]$ strain, uncovering an essential interplay between the two regions of the protein (Douglas et al., 2008). Intriguingly, an L94A mutation in the non-prion domain of Rnq1, which causes the protein to assemble into toxic but SDS soluble aggregates in a $[rnq^-]$ strain (Douglas et al., 2008), also induces mislocalization of Spc42 (Treusch and Lindquist, 2012). Thus, toxicity in a $[RNQ^+]$ strain may require the PrD to drive aggregation and the non-prion domain to mediate Spc42 interaction, both of which are essential for sequestration.

Together, these studies indicate that overexpression of Sup35 or Rnq1 in strains propagating their prion forms alters their interactions with their cellular environments. In the case of Sup35, its normal interaction with Sup45 is enhanced, presumably by mass action, to sequester this cellular factor in a non-functional form (Figure 1a) (Stansfield et al., 1995, Zhouravleva et al., 1995). In the case of Rnq1, the protein is not essential; its deletion has no known effects on yeast growth (Sondheimer and Lindquist, 2000, Strawn and True, 2006), and $[RNQ^+]$ strains grow normally when Rnq1 is expressed from its endogenous promoter (Derkatch et al., 1997). Thus, the Rnq1/Spc42 interaction is likely a gain-of-function event that may be explained by the propensity of proteins with high intrinsic disorder to engage in promiscuous interactions at elevated doses (Figure 1b) (Alberti et al., 2009, Cascarina and Ross, 2014, Vavouri et al., 2009). In either case, the imbalance brought about by overexpression converts benign protein aggregates into toxic species.

PolyQ Toxicity in Yeast

Given its experimental manipulability and the presence of endogenous amyloidogenic proteins, *Saccharomyces cerevisiae* has emerged as a powerful model for studying protein misfolding-related disease mechanisms. Particular effort has been focused on proteins containing polyQ repeats, including variants of the huntingtin (Htt) protein that are associated with Huntington's Disease (Group, 1993). In the case of Htt, a truncated protein encoded by exon I aggregates in yeast through a process that positively correlates with both the number of glutamines and the expression level of the protein (Krobitsch and Lindquist, 2000, Cao et al., 2001, Dehay and Bertolotti, 2006, Duennwald et al., 2006b). Intriguingly, overexpression of polyQ-expanded Htt in non-prion yeast strains leads to the accumulation of SDS-resistant aggregates of Sup35, Rnq1, and Pub1, another glutamine-rich protein, and polyQ-expanded Htt toxicity can be suppressed by deletions in polyglutamine and asparagine (polyQN)-rich proteins (Giorgini et al., 2005) or by expression antioxidant GPx enzymes which reduce ROS and presumably oxidatively damaged proteins (Mason et al., 2013). Together these observations suggest cellular limitations on the ability to control

aggregation of these metastable proteins (Kochneva-Pervukhova et al., 2012, Urakov et al., 2010). A particularly intriguing example of this effect is the toxicity of a synthetic poly-Q protein fused to GFP (pQ56-GFP), which leads to cell-cycle arrest due to compromised assembly of the septin complex at the yeast bud neck (Kaiser et al., 2013). This defect can be suppressed by conditions that limit the number of pQ56 aggregates by promoting the formation of larger complexes, such as deletion or inhibition of Hsp104 or Pho5, or by higher ploidy (Kaiser et al., 2013), which has been similarly shown to alter the accumulation of Sup35 aggregates in a [*PSI*⁺] by changing the prion:chaperone ratio (DiSalvo et al., 2011). Although not discussed in this study, two septin family members have been shown to form amyloid fibers *in vitro* (Garcia et al., 2007, Pissuti Damalio et al., 2012) and to associate with neurofibrillar tangles in Alzheimer's Disease (Kinoshita et al., 1998) and cytoplasmic inclusions in Parkinson's Disease (Ihara et al., 2003), raising the possibility that the limitations on protein quality control pathways in the presence of poly-Q expanded protein aggregates can cause septin aggregation and thereby toxicity.

In addition to cell-based limitations imposed by protein aggregation, more direct pathways to promote toxicity exist. For example, polyQ-expanded Htt aggregation and its associated toxicity are strongly enhanced by overexpression of polyQN-rich proteins or by the presence of the endogenous yeast prions [*RNQ*⁺] and [*PSI*⁺] (Meriin et al., 2002, Duennwald et al., 2006a, Kochneva-Pervukhova et al., 2012, Gong et al., 2012, Zhao et al., 2012, Gokhale et al., 2005, Giorgini et al., 2005), and this enhancement corresponds to co-localization of the aggregating proteins (Meriin et al., 2003, Duennwald et al., 2006a, Gong et al., 2012). Given the ability of polyQ-expanded Htt to induce aggregation of Sup35 (Kochneva-Pervukhova et al., 2012, Urakov et al., 2010) and the essential function of Sup35 in translation termination (Ter-Avanesyan et al., 1993), several groups explored the possibility of Sup35 sequestration as a mechanism for Htt toxicity in yeast (Gong et al., 2012, Kochneva-Pervukhova et al., 2012, Zhao et al., 2012). In the presence of [*RNQ*⁺] alone, polyQ-expanded Htt clearly induced aggregation of Sup35 (Gong et al., 2012, Kochneva-Pervukhova et al., 2012), but expression of the Sup35 functional domain was efficient in suppressing toxicity in one study (Kochneva-Pervukhova et al., 2012) but not in another (Gong et al., 2012). However, in the presence of both [*RNQ*⁺] and [*PSI*⁺], the toxicity of polyQ-expanded Htt is efficiently suppressed by expression of the functional domain of Sup35 (Gong et al., 2012, Zhao et al., 2012). Thus, while either type of aggregate alone is benign, the combination of polyQ and prion aggregates creates an imbalance presumably between the aggregation assembly pathway and cellular protein quality control pathways that promotes sequestration of Sup35 and thereby toxicity (Figure 1b).

In its native environment, Htt will not encounter a Sup35 homolog with a QN-rich domain (Jean-Jean et al., 1996). Nonetheless, similar types of interactions have been observed in patient-derived tissues and in cell and animal models of polyQ-expansion diseases. In the case of Htt, polyQ-expanded versions of the protein are known to induce co-aggregation of other proteins containing smaller glutamine-rich stretches of amino acids, such as the CREB binding protein (CBP) and the TATA binding protein (TBP), which do not aggregate on their own (McC Campbell et al., 2000, Perez et al., 1998, Chai et al., 2002, Kim et al., 2002, Steffan et al., 2000). Notably, these gain-of-function interactions clearly impact the

biological outcome of Htt aggregation: Htt toxicity in tissue culture and in mice can be suppressed by overexpression of CBP (Jiang et al., 2006, Nucifora et al., 2001), and the toxic interaction between Htt and human TBP, when reconstituted in yeast, can be suppressed by expression of the non-glutamine-rich yeast TBP (Schaffar et al., 2004). This sequestration model is likely to be more broadly generalizable, as synthetic amyloid-like proteins have interactome sizes that correlate directly with their toxicity in cell culture (Olzscha et al., 2011), and changes in the dosage of nearly 50% of the Htt interactome genetically modifies Htt toxicity *in vivo* (Kaltenbach et al., 2007). Thus, while there are certainly other potential mechanisms of polyQ-mediated toxicity including proteasome impairment (Bence et al., 2001) and membrane disruption (Arispe et al., 1993, Volles et al., 2001, Kremer et al., 2001), the sequestration and resulting functional titration of essential proteins by amyloid aggregates is a recurring theme in toxicity.

Aggregate Dynamics

Protein misfolding diseases most frequently correlate with the accumulation of aggregates, but studies in many systems suggest that pathogenesis is more accurately a function of the particular type(s) of aggregates present rather than the fraction of protein aggregated (Caughey and Lansbury, 2003, Haass and Selkoe, 2007). The emerging consensus suggests that soluble oligomers rather than high molecular weight complexes are the disease-causing culprits (Cohen et al., 2006, Saudou et al., 1998, Arrasate et al., 2004, Chesebro et al., 2005, Piccardo et al., 2007). However, studies in yeast suggest additional complexity in the link between aggregation and toxicity, particularly in the context of a sequestration model.

In yeast, the toxicity of an N-terminally flag-tagged Htt exon I fragment containing 103 glutamines (FHttQ103) is only observed in a $[RNQ^+]$ strain in the absence of an immediately adjacent proline-rich region (Duennwald et al., 2006b, Krobisch and Lindquist, 2000, Meriin et al., 2002). The toxicity of FHttQ103, however, can be suppressed by co-expression of a Htt exon I fragment containing 25 glutamines (HttQ25), but only if the latter contains the adjacent proline-rich region (HttQ25P) (Duennwald et al., 2006a, Wang et al., 2009). Because HttQ25P cannot aggregate on its own but does co-aggregate with FHttQ103, the proline-rich region likely acts in trans, when incorporated into FHttQ103 aggregates, to alleviate polyQ toxicity in yeast (Duennwald et al., 2006a, Wang et al., 2009).

Intriguingly, while an FHttQ103 variant containing the proline-rich region (FHttQ103P) is not toxic in a $[RNQ^+]$ strain (Duennwald et al., 2006a, Wang et al., 2009), this protein is toxic in a $[RNQ^+]$ $[PSI^+]$ strain, where the pool of functional Sup35 is already depleted (Gong et al., 2012, Zhao et al., 2012). Thus, although FHttQ103 can also induce toxicity through the sequestration of actin assembly proteins (Meriin et al., 2003), the synthetic interaction with $[PSI^+]$ suggests that FHttQ103 toxicity, like that of FHttQ103P, can arise through the sequestration of Sup35 but that the latter is less efficient in inactivating Sup35. Indeed, FHttQ103P and Sup35 co-localize *in vivo*, and FHttQ103P toxicity, like that of FHttQ103, is suppressed by expression of the functional domain of Sup35 (Gong et al., 2012, Zhao et al., 2012). But, the proline-rich region is unlikely to reduce toxicity simply by directly decreasing the affinity of Htt for Sup35 through this binary interaction, as HttQ25P acts dominantly (Duennwald et al., 2006a, Wang et al., 2009). Rather, the available

observations suggest that the proline-rich region, either in *cis* or in *trans*, mediates its effects by altering the dynamics of Htt aggregates *in vivo*. Specifically, both FHttQ103 and FHttQ103P form aggregates in a $[RNQ^+]$ strain, but the FHttQ103P aggregates are less SDS-resistant, larger in size and fewer in number (Dehay and Bertolotti, 2006, Wang et al., 2009, Duennwald et al., 2006a), attributes which may together restrict the binding promiscuity of aggregates by limiting their available interaction surfaces (Figure 1b). Importantly, the single foci formed by FHttQ103P localize to the spindle pole body and depend on microtubule activity, which suggest that smaller foci might form initially and then coalesce into a larger complex, known as an aggresome (Wang et al., 2009), as is the case in mammalian cells (Figure 1) (Wang et al., 2009, Johnston et al., 1998).

These relationships between aggregate dynamics and their biological outcomes are also observed for the yeast prions, but with the opposite correlation. Under moderate expression levels where the prion state is not toxic, both Sup35 and Rnq1 localize to multiple, highly mobile foci in the cytoplasm of $[PSI^+]$ and $[RNQ^+]$ strains, respectively (Satpute-Krishnan and Serio, 2005, Sondheimer and Lindquist, 2000). Upon their overexpression to toxic levels, Sup35 and Rnq1 accumulate in single immobile focus that co-localizes with Sup45 or Spc42, respectively, in the cytoplasm (Vishveshwara et al., 2009, Douglas et al., 2008, Kaganovich et al., 2008, Treusch and Lindquist, 2012). Thus, while the ability to sequester essential cellular proteins is impacted by the assembly state of the aggregation-prone proteins, there appears to be no single toxic species based on size. Rather, other properties of each aggregation-prone protein must necessarily determine which species participates in the toxic interactions.

Chaperone Limitations

While the overexpression of prions and polyQ-expanded proteins promotes their assembly into aggregates that are associated with toxicity, cellular quality control pathways exist to counterbalance this propensity (Hartl et al., 2011). Under conditions of normal expression, the accumulation and size of protein aggregates is a function of their assembly, disassembly, and dilution, through either degradation or transmission (Figure 1) (Sindi and Serio, 2009). Upon overexpression, the size of these complexes is likely to increase because assembly is enhanced, and the pathways that counteract this process become inefficient due to the stability of the aggregates and the limited capacity of cellular quality control pathways to clear them (Derdowski et al., 2010, DiSalvo et al., 2011, Sindi and Serio, 2009, Voisine et al., 2010).

Numerous studies have demonstrated that elevating chaperone levels can reduce the accumulation of aggregates of amyloidogenic proteins and reverse toxicity (Broadley and Hartl, 2009, Muchowski and Wacker, 2005), but chaperone proteins are also absolutely required for the accumulation and subcellular localization of these complexes and therefore impact toxicity through other routes. In the case of the yeast prions, a core group of molecular chaperones has been implicated in the disassembly pathway for these aggregates. The AAA⁺ ATPase Hsp104, which functions as a molecular disaggregase, and its co-chaperones Hsp70 (Ssa1/2) and Hsp40 (Sis1) collaborate to fragment prion aggregates into smaller complexes by extracting monomers (Chernoff et al., 1995, Higurashi et al., 2008,

Lum et al., 2004, Ness et al., 2002, Satpute-Krishnan et al., 2007, Tessarz et al., 2008, Tipton et al., 2008, Park et al., 2012). Hsp104 is also required for the accumulation of aggregates containing polyQ-expanded proteins in yeast (Cao et al., 2001, Dehay and Bertolotti, 2006, Kimura et al., 2004, Krobisch and Lindquist, 2000, Meriin et al., 2002). But, while Hsp104 has no known homolog in metazoans, members of both the Hsp70 and Hsp40 chaperone families have been implicated in the toxicity of polyQ expansions in yeast and in other model systems (Muchowski and Wacker, 2005, Kobayashi and Sobue, 2001), suggesting that these chaperone families retain the ability to recognize amyloidogenic proteins across evolutionary time.

Beyond promoting amyloid accumulation, chaperones impact amyloid interactions and localization. Despite their classification as “misfolded”, amyloid aggregates are actually highly ordered cross β structures (Eisenberg and Jucker, 2012). Nevertheless, *in vitro* amyloid fibers bind stably to dyes such as ANS, which also recognize molten globules, suggesting the exposure of hydrophobic regions in these structures that may be targeted by chaperones (Stryer, 1965, Schaffar et al., 2004, Serio et al., 2000). Consistent with this idea, the prion forms of Sup35 and Rnq1 form stable, stoichiometric complexes with the Hsp70 Ssa1/2 (2:1 ratio) and with the Hsp40 Sis1 (1:1 ratio), respectively, but because these chaperones are more abundant than the prion proteins, their interactions are not detrimental under normal expression conditions (Bagriantsev et al., 2008, Lopez et al., 2003, Sondheimer and Lindquist, 2000). However, at high doses, this balance is perturbed, leading to toxicity through multiple routes.

The toxicity associated with Rnq1 overexpression in a $[RNQ^+]$ strain can be suppressed by overexpression of Sis1, with which it forms a stable complex under normal expression conditions (Douglas et al., 2008, Lopez et al., 2003, Sondheimer and Lindquist, 2000). This observation suggests that Sis1 is a limiting factor in suppressing the toxicity of Rnq1 at high doses, and consistent with this idea, depletion of Sis1 promotes the toxicity of Rnq1 at lower doses in a $[RNQ^+]$ strain (Douglas et al., 2008, Lopez et al., 2003, Sondheimer and Lindquist, 2000). Intriguingly, an L94A mutation in Rnq1 reduces Sis1 binding, increases the toxicity of excess Rnq1 in a $[RNQ^+]$ strain, and promotes Rnq1 toxicity in a non-prion $[rnq^-]$ strain, where it now associates with Spc42 (Douglas et al., 2008, Treusch and Lindquist, 2012). Thus, Sis1 binding to Rnq1 may limit its binding promiscuity by shielding a hydrophobic interaction surface, as has been suggested for the suppression of HttQ72 toxicity by small heat shock protein proteins (sHsp) in yeast (Figure 1b) (Cashikar et al., 2005). But, the suppression of toxicity by overexpression of Sis1 can also be explained by the relocalization of Rnq1 to either the nucleus or to a cytoplasmic quality control body, where it is more efficiently assembled into SDS-resistant aggregates and is spatially separated from Spc42 (Figure 1c) (Douglas et al., 2009, Wolfe et al., 2013). In either case, limitations on the availability of Sis1 impact aggregate interactions with their cellular environment.

Chaperones have also been implicated in the toxic interactions between prion and polyQ-expanded Htt. Overexpression of either Ydj1 or Sis1, both members of the Hsp40 family, has no effect on the toxicity of FHttQ103 in a $[PSI^+]$ strain. However in a $[RNQ^+]$ strain, FHttQ103 toxicity is enhanced by Ydj1 overexpression and reduced by Sis1 overexpression

(Gokhale et al., 2005), and these effects correlate directly with changes in the accumulation of large FHttQ103 aggregates (Gokhale et al., 2005). The Hsp40-mediated changes in aggregate dynamics and toxicity is likely mediated by a competition between Rnq1 and FHttQ103 for these factors, as Rnq1 but not Sup35 aggregates have been shown to stably bind to Ydj1 and Sis1 at significant levels (Bagriantsev et al., 2008, Sondheimer et al., 2001, Summers et al., 2009a). Consistent with this idea, the suppression of FHttQ103 toxicity in a [RNQ⁺] strain by overexpression of Sis1 is dependent on Sis1 co-localization to Rnq1 aggregates (Wolfe et al., 2013), and overexpression of Ydj1 converts SDS-resistant Myc-HttQ53 aggregates to detergent-sensitive aggregates, likely through a direct interaction (Muchowski et al., 2000). Although the precise molecular mechanisms underlying these effects are still unknown, overexpression of Hsp40s ultimately alters aggregate dynamics, which presumably modulates their specific interactions with other cellular components and leads to toxicity.

Sis1 has also been implicated in suppressing the toxicity of polyQ-expanded Htt in the presence of non-prion misfolded proteins. In this case, expression of a myc-tagged Htt exon I fragment containing 96 glutamines and the adjacent proline-rich region (MHttQ96P) becomes toxic upon co-expression of a model misfolded protein (CG*) (Park et al., 2013). As is the case for Rnq1, MHttQ96P binds stably to Sis1, and overexpression of Sis1 is sufficient to reduce its toxicity in the presence of CG*, again suggesting a negative correlation between Sis1 availability and toxicity (Park et al., 2013). Rather than promoting promiscuous polyQ binding however, this Sis1 limitation is associated with an impairment of the 26S-dependent degradation of CG*, which requires its transport into the nucleus (Park et al., 2013). Sis1 shuttles between the nucleus and cytoplasm in response to stress and may mediate transport directly or perhaps indirectly through a modulation of the dynamics of cytoplasmic aggregates containing misfolded proteins (Figure 1c, d) (Park et al., 2013, Malinowska et al., 2012, Summers et al., 2013, Shiber et al., 2013). In either case, the sequestration of Sis1 through its stable interaction with Htt disrupts proteostasis.

The overexpression of polyQ-expanded Htt has also been implicated in the sequestration of other protein quality control factors including Cdc48 and its co-factors Npl4 and Ufd1. This sequestration is associated with defects in ER-associated degradation in yeast and in mammalian cells (Duennwald and Lindquist, 2008). But, these same factors have also been implicated in the assembly of HttQ103P into a single aggresome-like complex, which is associated with reduced toxicity in yeast (Figure 1c) (Wang et al., 2009). As was the case with Sis1, the balance between polyQ-expanded Htt and the Cdc48 system impacts both interactions and localization to mediate toxicity.

More generally, expression of polyQ-expanded proteins promotes the misfolding of non-amyloidogenic proteins with destabilizing mutations in *C. elegans* (Gidalevitz et al., 2006). These proteins are sufficiently buffered by protein quality control pathways in the absence of the polyQ protein to properly fold and function, suggesting a competition for an overlapping subset of factors (Gidalevitz et al., 2006). Consistent with this idea, the aggregation of a polyQ-expanded protein of intermediate length (40Q) was enhanced in *C. elegans* lines encoding metastable protein mutants. A component of this effect could result from the titration of co-translational quality control factors, such as the nascent polypeptide-

associated complex (NAC), which relocate from polysomes to polyQ aggregates and induce translational impairment presumably due to widespread protein misfolding (Figure 1d) (Kirstein-Miles et al., 2013).

Together, these studies suggest that while the chaperone-based aspects of protein quality control are insufficient to clear amyloid aggregates once they are established, these highly ordered structures are recognized as aberrant by the same pathways. The binding of chaperones to these complexes modulates their toxicity by shielding hydrophobic surfaces and impacting localization, which together alter their interactions (Figure 1b, c). When the balance between the levels of the aggregation-prone proteins and chaperones is altered by overexpression of the former, chaperones become limiting, exposing the amyloid to promiscuous interactions and jeopardizing other cellular processes that are dependent on chaperone interaction (Figure 1).

Conclusion

Studies in model organisms based on overexpression of prion and polyQ-expanded amyloid proteins have uncovered emerging complexity in the links between protein aggregation and toxicity. While a clear and unquestionable connection between protein misfolding and pathogenesis exists, specific attributes of the proteins and their expression levels promote toxicity through a variety of distinct events that have the common foundation of altered interactions. Despite artificial aspects of these systems, the lessons learned through them are likely to be relevant to pathogenesis under conditions of normal expression. Aggregates accumulate spontaneously in the absence of overexpression, suggesting inherent limitations on protein quality control pathways (Voisine et al., 2010). Moreover, the pathways maintaining proteostasis under normal conditions become impaired with age, creating new thresholds for the acquisition and biological impact of protein aggregates (Broadley and Hartl, 2009, David et al., 2010). Nonetheless, the existence of benign and functional amyloids and the fact that some of these species can be converted to toxic forms under conditions of overexpression suggests that the pathogenic progression of related proteins in mammals may be reversible through manipulations that seek to restore balance, even if these efforts fall short of aggregate clearance (Lindquist and Kelly, 2011).

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Figure 1.

Models for Prion and PolyQ Toxicity at High Doses. Natively folded prion/polyQ protein (blue stick and ball) converts to the amyloid form (corkscrew and ball) by associating with and elongating existing aggregates. These complexes are fragmented by chaperones (hexagons) but cannot be cleared under normal conditions. Both natively folded and amyloid-form protein engage in specific interactions with functional partners (ovals). At higher doses (below dotted line), the number of aggregates increases, and these complexes can coalesce into larger aggresomes depending on the properties of the aggregating protein and the availability of cellular factors. Under these conditions, (a) functional partners may be sequestered by mass action and/or chaperone limitations; (b) promiscuous interactions with normally non-binding partners (stars) may arise; (c) aggregates may mislocalize due to chaperone limitations; and/or (d) other cellular functions dependent on chaperone activity such as degradation and folding may become compromised due to their sequestration by aggregates. A color version of this figure is available online.