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MINI-REVIEW

Aggregation and degradation scales for prion-like domains: sequence features and context weigh in

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Abstract

Protein aggregation in vivo is generally combated by extensive proteostatic defenses. Many proteostasis factors specifically recognize aggregation-prone features and re-fold or degrade the targeted protein. However, protein aggregation is not uncommon, suggesting that some proteins employ evasive strategies to aggregate in spite of the proteostasis machinery. Therefore, in addition to understanding the inherent aggregation propensity of protein sequences, it is important to understand how these sequences affect proteostatic recognition and regulation in vivo. In a recent study, we used a genetic mutagenesis and screening approach to explore the aggregation or degradation promoting effects of the canonical amino acids in the context of G-rich and Q/N-rich prion-like domains (PrLDs). Our results indicate that aggregation propensity scales are strongly influenced by the interplay between specific PrLD features and proteostatic recognition. Here, we briefly review these results and expand upon their potential implications. In addition, a preliminary exploration of the yeast proteome suggests that these proteostatic regulation heuristics may influence the compositional features of native G-rich and Q/N-rich domains in yeast. These results improve our understanding of the features affecting the aggregation and proteostatic regulation of prion-like domains in a cellular context, and suggest that the sequence space for native prion-like domains may be shaped by proteostatic constraints.

Keywords Proteostasis · Protein aggregation · Prion · Prion-like · Protein degradation · Low complexity domain

Introduction

All cells depend on a suite of properly folded proteins for survival. While protein aggregation is not always detrimental [and in some cases may even be beneficial (Chakravarty and Jarosz 2018)], errors in protein folding often lead to toxic protein aggregation. This aggregation underlies a number of neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, among others (for review, see Brundin et al. 2010; Holmes and Diamond 2012; King et al. 2012; Costanzo and Zurzolo 2013; Li et al. 2013; Ling et al. 2013; Ramaswami et al. 2013; Taylor et al. 2016). Consequently, cells possess a molecular battalion of proteostasis factors involved in the detection and elimination of protein folding threats. Yet a number of proteins effectively evade the proteostasis machinery and form aggregates, suggesting that some proteins possess inherent features that simultaneously confer high aggregation propensity and the ability to evade proteostatic systems. Therefore, defining these sequence characteristics would advance our understanding of protein aggregation in vivo.

In yeast, a number of proteins can form self-perpetuating, infectious protein aggregates known as "prions". The protein domains responsible for this activity have unusual amino acid compositions which, at least in some cases, is the predominant feature facilitating prion aggregation (Ross et al. 2004, 2005). In accordance with these observations, the development of prion prediction methods have focused predominantly on amino acid composition to identify proteins with "*pr*ion-*l*ike *d*omains" (PrLDs) in the yeast and human proteomes (Alberti et al. 2009; Toombs et al. 2010; King et al. 2012; Espinosa Angarica et al. 2013; Lancaster et al. 2014; Afsar Minhas et al. 2017). Many of the proteins with top-scoring PrLDs have been associated with aggregation

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and neurodegeneration/myopathy in humans (for review, see King et al. 2012; Harrison and Shorter 2017). However, many proteostasis factors specifically recognize aggregation-prone protein features and either re-fold or degrade these proteins (Flynn et al. 1991; Rudiger et al. 1997a, b, 2001; Fredrickson et al. 2011, 2013; Willmund et al. 2013; Karagoz et al. 2014; Saio et al. 2014; Karagöz and Rüdiger 2015). The ability of yeast prion proteins to aggregate without triggering a strong proteostatic response makes them excellent models to explore the interplay between aggregation and proteostatic regulation.

Sequence features driving aggregation versus degradation of PrLDs

In a recent study, we explored the features of aggregation-prone proteins that lead either to their detection and degradation by the ubiquitin–proteasome system, or to the formation of self-propagating prion aggregates (Cascarina et al. 2018). Although canonical yeast prion domains tend to be extremely Q/N-rich, a number of the human PrLDs (while still moderately Q/N-rich) tend to be more G-rich. Might these compositional differences affect the molecular fates of aggregation-prone Q/N-rich and G-rich proteins in the eukaryotic intracellular environment?

We chose a canonical Q/N-rich yeast *p*rion *d*omain (PD) from Sup35, and two related G-rich human PrLDs from hnRNPA1 and hnRNPA2B1 as model substrates to examine how changes in the amino acid composition of Q/N-rich and G-rich PrLDs affect prion aggregation and degradation. When substituted in the place of a portion of the Sup35 PD, the hnRNPA1 and hnRNPA2B1 PrLDs are able to support prion activity in a mutation-dependent manner (Kim et al. 2013; Paul et al. 2017). We, therefore, randomly mutagenized a segment of the G-rich PrLDs within these fusion proteins in a manner analogous to previous mutagenesis of the Sup35 PD (Toombs et al. 2010; MacLea et al. 2015). Interestingly, while non-aromatic hydrophobic residues (I, L, M, and V) strongly promote prion formation in the Sup35 PD (Toombs et al. 2010; MacLea et al. 2015), the same residues led to rapid, proteasome-mediated degradation of the G-rich PrLDs. However, when the degradation-promoting sequences were substituted into the Q/N-rich Sup35 PD, they did not noticeably accelerate degradation. Progressively increasing the number of hydrophobic residues led to a step-wise increase in the degradation rate of the G-rich PrLDs before exceeding the resolution of our degradation assays, whereas progressively increasing hydrophobic content in an identical manner in the Sup35 PD led to a step-wise increase in the frequency of spontaneous prion formation. The Q/N content of the Sup35 was critical for resisting the degradation-promoting effects of hydrophobic residues: step-wise substitutions of G residues for Q/N residues in the Sup35 PD led to a step-wise increase in degradation rate when degradation-promoting sequences were present.

These results indicated that high Q/N content may be one feature that effectively overrides the otherwise degradation-promoting effects of the non-aromatic hydrophobic residues, allowing evasion of the ubiquitin–proteasome defenses. Conversely, the G-rich PrLDs are susceptible to enhanced proteolysis when nudged by certain aggregation-promoting residues.

We next asked whether any residues provide a sufficient balance between low detection by the ubiquitin–proteasome system and high aggregation propensity to facilitate prion aggregation of the G-rich PrLDs. After excluding the isolates with a degradation phenotype from our original phenotypic tests, we screened the remaining isolates for the ability to form rare yet stable prion aggregates. Aromatic residues were significantly over-represented among the identified [*PRION*$^{+}$] isolates, suggesting that they were simultaneously aggregation-prone and poorly detected by the ubiquitin–proteasome system in the context of the G-rich PrLDs.

Conclusions and perspectives

These observations illuminate multiple ways through which some aggregation-prone proteins may violate conventional wisdom regarding their proteostatic regulation, and highlight important caveats when thinking about protein aggregation in a cellular context. Although a tight link between exposure of aggregation-prone residues and efficient re-folding or clearance of the substrate by the proteostasis machinery has been well-documented (Flynn et al. 1991; Rudiger et al. 1997a, b, 2001; Fredrickson et al. 2011, 2013; Willmund et al. 2013; Karagoz et al. 2014; Saio et al. 2014; Karagöz and Rüdiger 2015), we find that this relationship is not absolute, and can be severed in at least two ways. First, Q/N-rich regions effectively buffered the degradation-promoting effects of hydrophobic residues, thereby permitting aggregation (Fig. 1a top). Second, aromatic residues promoted aggregation of the G-rich PrLDs without efficiently alarming the ubiquitin–proteasome system (Fig. 1a bottom). Therefore, while scales describing aggregation-propensity or degradation-propensity for the 20 canonical amino acids represent essential advances in our understanding of molecular biochemistry, these scales are heavily dependent on both the molecular and cellular contexts (Cascarina et al. 2017, 2018; Paul et al. 2017).

Ideally, our proteostatic defenses would be calibrated to recognize any protein whose aggregation propensity exceeds the solubility threshold under normal cellular conditions (Fig. 1b). However, it is interesting to note that both G-rich and Q/N-rich proteins showed gaps between aggregation and

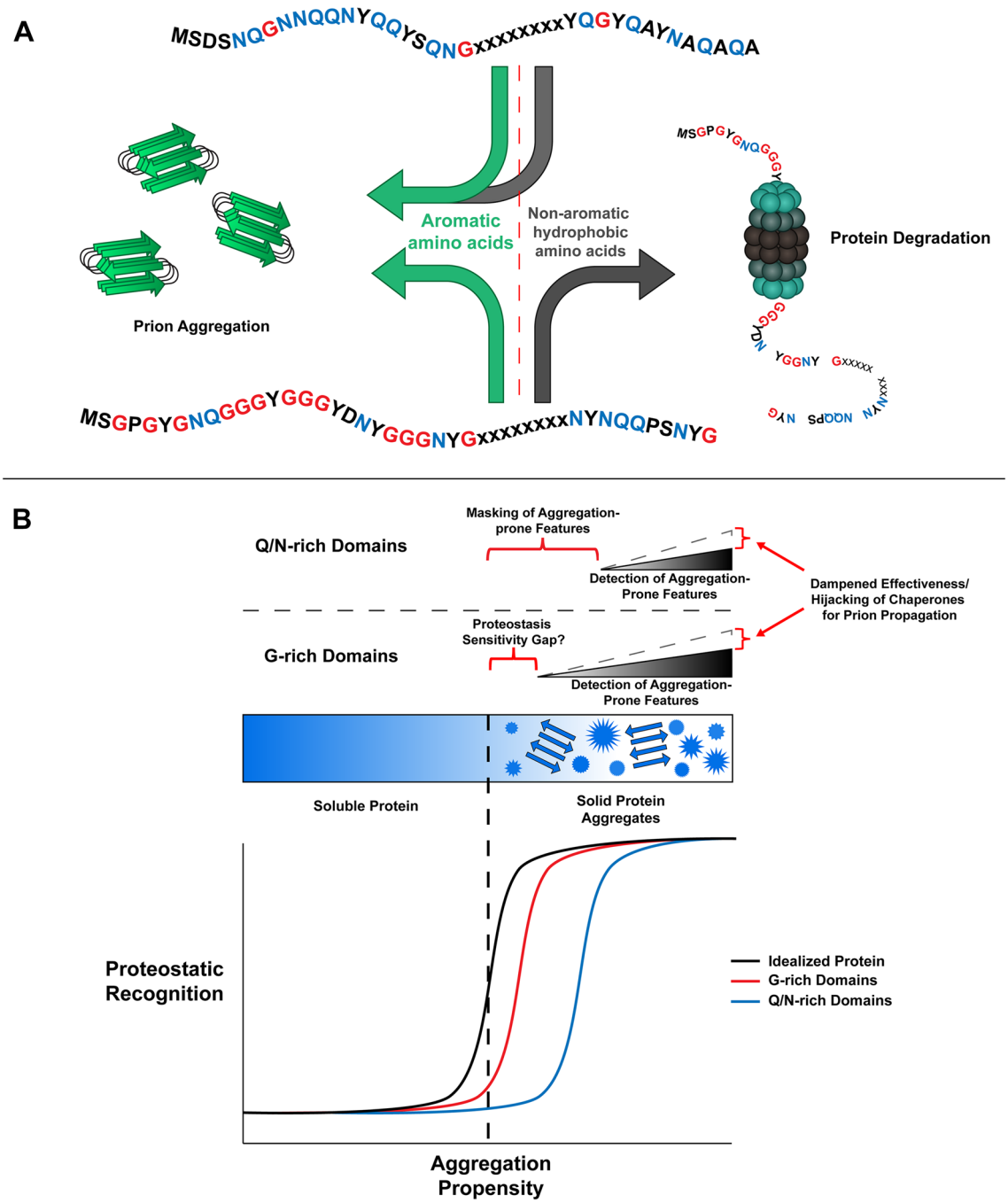

Fig. 1 The effects of molecular and cellular contexts on aggregation or degradation of PrLDs in vivo. **a** Aromatic and non-aromatic hydrophobic residues are strongly prion-promoting in the context of a Q/N-rich PD. By contrast, only aromatic amino acids retain their prion-promoting effects in the context of two related G-rich PrLDs, whereas non-aromatic hydrophobic residues accelerate their degradation via the proteasome. **b** A simplified hypothetical model depicting the interplay between intracellular counter-aggregation defenses and aggregation propensity of Q/N-rich and G-rich domains

proteostatic degradation (Fig. 1b). Although single-amino acid substitutions were sufficient to cause prion aggregation of G-rich PrLDs in our system, and are linked to neuromuscular degeneration in humans, these single substitutions were not sufficient to trigger degradation. One possible explanation is that the proteostasis machinery must balance sensitivity toward exposed hydrophobicity with specificity toward misfolded proteins. Exposure of a single hydrophobic residue within a G-rich domain may increase the aggregation propensity yet still lie below the sensitivity of relevant

proteostasis factors, effectively resulting in a proteostasis sensitivity gap for G-rich domains. For Q/N-rich PrLDs, this gap between aggregation and degradation may be even larger due to the masking of aggregation-prone features. Additionally, many yeast prions are actually able to propagate precisely because of proteostasis factors (predominantly Hsp104, along with Hsp40 and Hsp70 chaperones) attempting to disassemble existing aggregates (for review, see Chernova et al. 2017b). Therefore, these proteins use a combination of proteostasis evasion and hijacking strategies to aggregate and persist in vivo, potentially explaining why so many of these proteins are linked to functional and pathogenic aggregation.

This study provides a solid foundation for exciting new avenues of research. Although our G-rich PrLDs and Q/N-rich PD allowed us to explore the features controlling their behavior in great depth, it is important to note that these experiments were performed on just three model proteins; therefore, additional experiments will be required to determine to what extent these heuristics apply to other native G-rich and Q/N-rich domains. Surprisingly, although G-rich domains appear to be remarkably susceptible to proteolysis upon insertion of only a few hydrophobic residues (Cascarina et al. 2018), native yeast proteins with G-rich domains are actually associated with higher protein half-lives (i.e., lower degradation rates) and higher protein abundances relative to non-G-rich proteins (Cascarina and Ross 2018). How, then, do native yeast G-rich domains avoid extremely short half-lives despite their susceptibility to proteolysis?

A simple bioinformatic exploration of native yeast G-rich and Q/N-rich domains provides a possible explanation for this apparent discrepancy, and yields a number of interesting observations (Fig. 2). First, strongly degradation-promoting residues (ILMV) are strikingly underrepresented among G-rich domains, suggesting that the natural avoidance of degradation-promoting residues (perhaps via strong selective pressure at the molecular level) may contribute to the higher half-lives associated with proteins containing G-rich domains. By contrast, Q/N-rich domains tend to have a higher relative ILMV content, which may be facilitated by the ability of Q/N-rich domains to suppress the degradation-promoting effects of these residues. Second, strongly prion-promoting residues (FWY) are more common among G-rich domains compared to the degradation-promoting residues, suggesting that many native G-rich domains still possess at least some aggregation-prone features, which may enable some G-rich PrLDs to exhibit prion-like activity (Kim et al. 2013; Molliex et al. 2015; Xiang et al. 2015; Lee et al. 2016). Finally, native Q/N-rich domains tend to have fewer aromatic residues compared to the non-aromatic hydrophobic residues. This is somewhat surprising, given that the Q/N-rich prion domains from canonical yeast prion proteins tend to exhibit secondary biases for aromatic residues (particularly tyrosine; Harrison and Gerstein 2003; MacLea et al. 2015). However, when Q/N-rich domains from the known yeast prion proteins (Wickner 1994; Sondheimer and Lindquist 2000; Derkatch et al. 2001; Du et al. 2008; Alberti et al. 2009; Patel et al. 2009; Halfmann et al. 2012; Suzuki et al. 2012; Chernova et al. 2017a) are considered as a separate class, these domains exhibit lower ILMV and higher FWY content relative to Q/N-rich domains as a unified class. Interestingly, our lab has also found that, while both aromatic and hydrophobic residues within Q/N-rich prion domains strongly promote prion nucleation, the aromatic residues appear to uniquely facilitate prion propagation in addition to nucleation (MacLea et al. 2015). This may suggest that aromatic residues provide a sufficient balance between aggregation propensity and aggregate heritability over generations (both of which are necessary for many of the known prions to act as infectious proteins in yeast), and could explain, at least in-part, why some proteins with Q/N-rich domains are aggregation-prone, yet do not appear to form stable prions in vivo (Alberti et al. 2009; MacLea et al. 2015).

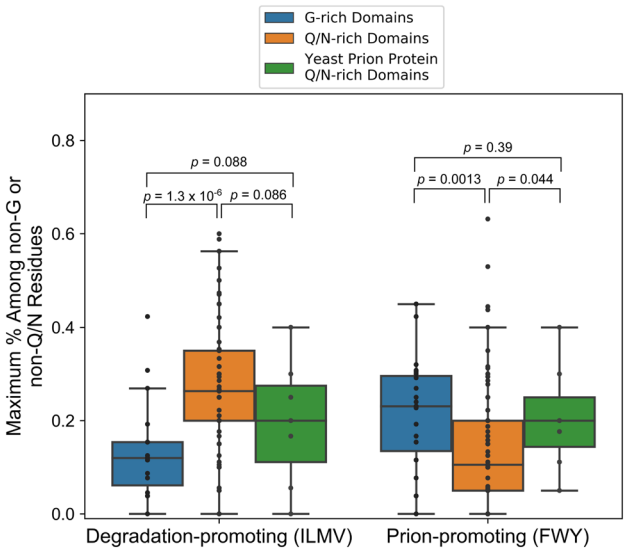

Fig. 2 Maximum percentage of degradation-promoting or aggregation-promoting residues within native yeast G-rich and Q/N-rich domains. The yeast proteome was scanned exhaustively using a 40 amino acid sliding window to identify domains with ≥35% G or ≥50% Q/N (the approximate G or Q/N compositions of the hnRNP PrLDs and Sup35 nucleation domain, respectively). Box plots represent the maximum percentages of degradation-promoting (ILMV; left) or prion-promoting (FWY; right) residues among non-G residues within G-rich domains or non-Q/N residues within Q/N-rich domains. For the yeast prion proteins with Q/N-rich domains, maximum percentage values for the known Q/N-rich yeast prion proteins were simply extracted from the Q/N-rich domain dataset and represented as independent box plots. Of the nine canonical yeast prion proteins, seven have Q/N-rich domains that pass our Q/N threshold (Cyc8, Rnq1, Sup35, Lsb2/Pin3, Swi1, Ure2, and Mot3). Plotting and statistics were performed with the Matplotlib and SciPy packages, respectively

It is also important to note that our results are not incompatible with an age-related decline in proteostatic quality control: it may be that some of the aggregation-prone proteins are effectively held at bay until a deterioration in the cell's proteostatic defenses. Proteins on the precipice of aggregation, yet effectively recognized by a healthy proteostasis network, may become toxic when defenses are down (Chernova et al. 2017b; Klaips et al. 2018; Wisniewski et al. 2018). Finally, not all protein aggregation is deleterious. Many proteins naturally form amyloid fibers (Ryzhova et al. 2018), and controlled protein aggregation has been shown, in many cases, to mediate a variety of beneficial processes (Chuang et al. 2018; Chakravarty and Jarosz 2018). Proteins involved in beneficial protein aggregation may possess sequence features that allow evasion of the proteostasis machinery.

Collectively, these studies indicate that the sequence landscapes of prion and prion-like domains that facilitate aggregation in vivo are strongly influenced by both the intramolecular context (i.e., the composition of neighboring protein regions) and the intracellular context (including, but perhaps not limited to, the native proteostasis factors). The apparent fluidity of aggregation- and degradation-propensity scales for the 20 canonical amino acids may necessitate the development of multiple scales in different molecular contexts (i.e., prion-like domains with varying starting compositions), and validation of these scales in vivo, where proteostasis systems can sway protein fates. Our results highlight the importance of considering proteostatic regulation of PrLDs in addition to inherent aggregation propensity, and enhance our understanding of both beneficial and pathological protein aggregation.

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