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The [*RNQ*$^+$] prion
A model of both functional and pathological amyloid

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Abbreviations: PFD, prion-forming domain; Q/N-rich, glutamine and asparagine rich; polyQ, polyglutamine; s.d., single dot; m.d., multi dot; G/F, glycine and phenylalanine domain; G/M, glycine and methionine domain

The formation of fibrillar amyloid is most often associated with protein conformational disorders such as prion diseases, Alzheimer disease and Huntington disease. Interestingly, however, an increasing number of studies suggest that amyloid structures can sometimes play a functional role in normal biology. Several proteins form self-propagating amyloids called prions in the budding yeast *Saccharomyces cerevisiae*. These unique elements operate by creating a reversible, epigenetic change in phenotype. While the function of the non-prion conformation of the Rnq1 protein is unclear, the prion form, [*RNQ*$^+$], acts to facilitate the de novo formation of other prions to influence cellular phenotypes. The [*RNQ*$^+$] prion itself does not adversely affect the growth of yeast, but the overexpression of Rnq1p can form toxic aggregated structures that are not necessarily prions. The [*RNQ*$^+$] prion is also involved in dictating the aggregation and toxicity of polyglutamine proteins ectopically expressed in yeast. Thus, the [*RNQ*$^+$] prion provides a tractable model that has the potential to reveal significant insight into the factors that dictate how amyloid structures are initiated and propagated in both physiological and pathological contexts.

Introduction

Protein conformational disorders are associated with the misfolding and aggregation of proteins into cross-β-sheet structures called amyloid.[1] Interestingly, these disorders may result from a common underlying mechanism that has been described as prion-like.[2] Prions are proteins that form a self-propagating, amyloid-like structure that converts protein from its native state into the prion conformation.[3] When the mammalian protein PrP misfolds to its prion conformation, it is infectious. This infectious prion protein is the causative agent of one class of mammalian protein conformational disorders called transmissible spongiform encephalopathies. While the term amyloid is traditionally used in the context of cellular dysfunction and disease, many examples of amyloid structures with normal cellular functions are emerging (reviewed in refs. 4–6). Hence, elucidating how amyloid structures form and propagate is critical to understanding disease pathogenesis and determining how this type of folded structure can have a physiological function.

Much information regarding amyloid formation has been gleaned from the yeast model system and the range of tools it provides. Several unrelated proteins have been demonstrated to form prions in yeast. Yeast prions are associated with changes in phenotype that are inherited epigenetically, thereby showing how amyloid structures can provide a means of regulating cellular functions and phenotypes. Three well-studied yeast prions are [*PSI*$^+$], [*URE3*] and [*RNQ*$^+$], formed by the proteins Sup35, Ure2 and Rnq1, respectively. When the essential translation termination factor Sup35p is sequestered into aggregates in [*PSI*$^+$] cells, it alters translation termination and acts as an omnipotent nonsense suppressor.[7,8] [*PSI*$^+$] provides growth advantages under certain conditions and as such, it is interesting to consider how this type of element could impact the ability of yeast to adapt to changing environments and ultimately govern the evolution of new traits.[9-16] When the transcriptional regulator Ure2p forms the [*URE3*] prion, the transcription of genes involved in nitrogen catabolism is derepressed.[17] This allows the cell to utilize poor nitrogen sources in the presence of good nitrogen sources.

In contrast to Sup35p and Ure2p, the physiological function of the Rnq1 protein has yet to be determined. The [*RNQ*$^+$] prion, however, is required for the de novo formation of both [*PSI*$^+$] and [*URE3*], and was originally classified as the [*PIN*$^+$] element, for [*PSI*$^+$] inducible.[18-22] The [*RNQ*$^+$] prion has been found in wild yeast isolates,[23,24] suggesting that it might not be detrimental in many genetic backgrounds and growth conditions and instead may play some functional role. While [*PSI*$^+$] and [*URE3*] were discovered by phenotype, Rnq1p was identified as a putative prion protein by analyzing the yeast proteome for sequences similar to the glutamine and asparagine (Q/N)-rich prion-forming domains (PFDs) of Sup35p and Ure2p.[25] These domains are defined as the regions that are both necessary and sufficient for prion formation.[26] Definitive confirmation of the prion properties of Rnq1p was shown by transforming in vitro generated Rnq1p-PFD fibers (purified, recombinant Rnq1p-(132–405)) into [*rnq*$^-$] yeast to convert cells to [*RNQ*$^+$].[27,28] Like other yeast prion proteins, as well as those associated with protein conformational disorders, Rnq1p has the propensity to form amyloid in

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vitro with parallel in-register cross-β-sheets.[29] Strikingly, prion proteins can form several unique prion variants (or strains) that have slight differences in their β-sheet structure that constitute distinct amyloid conformations.[30,31] Such different structures are presumably the underlying cause of the diverse phenotypic variation seen in both yeast and in prion diseases.[22,32-34] In the case of Rnq1p, several different [*RNQ*$^+$] variants have been described, and these correspond to different levels to which they facilitate the formation of [*PSI*$^+$].[22]

In this review, we summarize the properties and protein interactions of the [*RNQ*$^+$] prion and highlight its similarities and differences to other yeast prions. This discussion provides a framework to study how [*RNQ*$^+$] may represent another example of a functional amyloid. In addition, we describe how [*RNQ*$^+$] can be used to model pathological amyloid, thereby showing how the same protein may form both toxic and non-toxic aggregates.

The PFD of Rnq1p is Complex and May Not be Confined to the Q/N-Rich Region

The N-domain of Rnq1p may be involved in [*RNQ*$^+$] maintenance. While most polypeptides may be able to form amyloid structures given the right conditions, there are a number of intrinsic factors that determine whether a protein will aggregate under physiological conditions in vivo.[35] A high degree of hydrophobicity, minimal net charge, and an intrinsically disordered region often contribute to the propensity of many amyloidogenic proteins, such as PrP and the Alzheimer disease protein Aβ, to form β-sheets and aggregate.[1] In contrast, polyglutamine (polyQ) proteins are characterized by having a long, highly polar stretch of glutamine residues.[36] The PFDs of many yeast prion proteins are similarly polar as they are enriched in glutamines and asparagines.[26] Thus, our understanding of how amino acid composition influences aggregation and amyloid formation is incomplete.

The PFD of Rnq1p was initially defined as the C-terminal Q/N-rich region (aa 153–405) by sequence analysis using the PFDs of Sup35p and Ure2p, and this was later verified experimentally.[25] The N-terminal domain (aa 1–152) of Rnq1p, on the other hand, has no known function. To better understand the regions of Rnq1p that are important for [*RNQ*$^+$] propagation, the effect of a series of *RNQ1* truncations on [*RNQ*$^+$] propagation was tested.[37] With the exception of one construct (aa 172–405) that transmitted [*RNQ*$^+$] more efficiently than the entire PFD (aa 133–405 in this case), the efficiency of propagation decreased with decreasing fragment lengths. This result provided the first indication that, in addition to the PFD, the N-domain may be involved in maintenance of the [*RNQ*$^+$] prion.

Other studies have also revealed a potential role for the N-domain in the propagation of [*RNQ*$^+$]. An interaction between Rnq1p and the Hsp40 Sis1p is required for [*RNQ*$^+$] propagation.[38] Mutation of one residue in Rnq1p in the putative Sis1p binding site (L94A) disrupts this interaction and eliminates the [*RNQ*$^+$] prion.[39,40] This suggests that the N-terminus of Rnq1p facilitates the interaction with Sis1p to maintain [*RNQ*$^+$]. Additionally, *rnq1* alleles having missense mutations in the N-terminus of Rnq1p destabilize [*RNQ*$^+$] propagation,[41,42] further supporting the notion that this domain is involved in [*RNQ*$^+$] propagation. Interestingly, however, these *rnq1* mutants do not disrupt the interaction with Sis1p,[41] suggesting that the N-terminal region may contribute functions beyond interaction with Sis1p. Finally, in a screen aimed to identify residues in the Rnq1p-PFD important for [*RNQ*$^+$] propagation, none of the PFD mutants impaired [*RNQ*$^+$] when tested in the context of full-length Rnq1p,[43] and only one mutation in the PFD that affects [*RNQ*$^+$] propagation has since been found.[41] Collectively, these data yield conflicting results as to whether the N-terminus of Rnq1p acts as a positive or negative regulator in the maintenance of the [*RNQ*$^+$] prion. Elucidating the role of the N-domain will provide insight into how other intragenic or non-PFD regions may be involved in the formation and propagation of amyloid structures in a pathological or physiological setting.

The Rnq1p-PFD is comprised of multiple recognition elements that act cooperatively. In addition to regions outside of the Rnq1p-PFD being involved in [*RNQ*$^+$] propagation, it seems clear that the Rnq1p-PFD is more complex than the PFDs of Sup35p or Ure2p. The Sup35p-PFD, for example, is much smaller (aa 1–123) with two defined regions important for [*PSI*$^+$] propagation: a short Q/N-rich tract (aa 5–27) and a region of clearly defined oligopeptide repeats (aa 41–95).[33,44] These regions can be functionally separated into sequences important for prion formation and sequences important for prion propagation.[45] Likewise, the oligopeptide repeats in the mammalian prion protein, PrP, are involved in dictating prion infectivity.[46-48] In contrast, Ure2p does not have any oligopeptide repeats and the Rnq1p-PFD has loosely defined oligopeptide repeats among four largely separated Q/N-rich regions.[26] Hence, the regions that influence the propagation and heritability of Rnq1p aggregates remain poorly defined. None of the four Q/N-rich regions of the Rnq1p PFD is sufficient to maintain [*RNQ*$^+$].[49] The presence of either QN2 (aa 218–263) or QN4 (aa 337–405) is required, but the presence of QN1 (aa 185–198) or QN3 (aa 279–319) strongly enhances propagation.[49] Thus, the Rnq1p-PFD has multiple sequence determinants that cooperate to propagate [*RNQ*$^+$], thereby creating a complex, composite PFD with ill-defined roles for each of these sequence elements. Defining these roles will help us understand how the primary sequence of a protein is involved in dictating amyloid formation.

[*RNQ*$^+$] Propagation Depends on Interactions with Chaperones

Differential roles of chaperones on prion propagation. A conserved network of molecular chaperones helps proteins adopt and maintain their proper fold, thereby combating the misfolding and aggregation of proteins that can cause disease.[50] Chaperones also play a major role in the propagation of all yeast prions.[51-53] Proteins in the Hsp40 and Hsp70 families deliver aggregated substrates to the disaggregase Hsp104p for resolubilization.[54,55] This process serves to fragment prion aggregates into seeds that can be efficiently transmitted from mother to daughter cells during mitosis.[56-58] Both Hsp104p and the essential Hsp40 Sis1p are required

for the propagation of [*PSI*$^+$], [*URE3*] and [*RNQ*$^+$].[25,38,59-61] Several lines of evidence, however, suggest that these chaperones may be differentially involved in propagating these prions. [*PSI*$^+$], [*URE3*] and [*RNQ*$^+$] show distinct sensitivities to both expression levels and mutation of Sis1p and Hsp104p.[40,60,62,63] The overexpression of Hsp104p eliminates [*PSI*$^+$], but does not affect propagation of [*RNQ*$^+$] or [*URE3*].[8,38,59,61] In contrast, [*RNQ*$^+$] and [*URE3*] are more sensitive to Sis1p levels than [*PSI*$^+$]. Sis1p overexpression does not affect [*RNQ*$^+$] propagation,[38] but when Sis1p is downregulated, both [*RNQ*$^+$] and [*URE3*] are lost within 20 generations as compared to >60 generations that it takes to lose [*PSI*$^+$].[60,64] When Hsp104p is inhibited by guanidine hydrochloride, though, the rate of loss is similar for all three prions and is comparable to the loss of [*RNQ*$^+$] and [*URE3*] when Sis1p is downregulated.[60] One possible explanation as to why these prions show different sensitivities to Sis1p is that one of the other 12 Hsp40s present in yeast could partially compensate for Sis1p in [*PSI*$^+$] propagation. However, none of the other Hsp40s are required for propagation of [*PSI*$^+$], [*URE3*] or [*RNQ*$^+$].[60,65] Besides Sis1p, the only other Hsp40 shown to bind Rnq1p is Ydj1p.[65,66] Ydj1p is dispensable for [*RNQ*$^+$] propagation,[65] but the overexpression of Ydj1p cures some variants of [*RNQ*$^+$].[22] Such differences in chaperone requirements of [*RNQ*$^+$], [*PSI*$^+$] and [*URE3*] may indicate that the structural differences between different prions and/or variants are responsible for dictating how chaperones recognize or fragment prion aggregates. Our understanding of this highly specific interplay between molecular chaperones and prions is far from complete. Additional mechanistic studies are required to better understand how chaperones regulate prion propagation—an understanding that could lead to therapeutic development for protein conformational disorders.

Sis1p and [*RNQ*$^+$] propagation. Sis1p binds Rnq1p in equimolar ratios in [*RNQ*$^+$] cells and this interaction is required for [*RNQ*$^+$] propagation.[38-40,65] Both the human homolog of Sis1p, HDJ1, and the Drosophila homolog, DROJ1, can compensate for the loss of Sis1p in viability and [*RNQ*$^+$] propagation, suggesting a conserved function of Sis1p acts in prion propagation.[65] The sequences of Class I and Class II Hsp40s were compared to analyze the specificity of Sis1p in prion propagation.[65] Both types of Hsp40s have an N-terminal J-domain that mediates their interaction with Hsp70s and stimulates Hsp70 ATPase activity.[67] A glycine-rich region is adjacent to the J-domain in both classes as well. In Sis1p, this domain is divided into two parts: a G/F domain rich in glycine and phenylalanine residues and a G/M domain rich in glycine and methionine. The function of these domains is unclear. Following the glycine-rich domain, Class I Hsp40s have a cysteine-rich domain that is not present in Class II Hsp40s like Sis1p. Lastly, both classes have a C-terminal domain (CTD) that can bind unfolded substrates in vitro.[68] The G/F domain is the most critical part of Sis1p in [*RNQ*$^+$] propagation as its deletion eliminates [*RNQ*$^+$], although the G/M domain and the CTD are also likely to be involved as deletion of these domains alters aggregates of Rnq1p.[38] The dependence of [*RNQ*$^+$] on the G/F domain helps explain the specificity of Sis1p, as this region is fairly unique among Hsp40s. The region of aa 101–113 in the Sis1p G/F domain is not present in other Hsp40s, and is required for [*RNQ*$^+$] propagation: two point mutations in this region (N108I and D110G) impair maintenance of [*RNQ*$^+$].[65] Interestingly, in vitro, Sis1ΔG/F can still bind substrates and stimulate ATPase activity of the Hsp70 Ssa1p,[69] which can interact with Rnq1p.[38] This suggests that these functions of Sis1p are dispensable for [*RNQ*$^+$] propagation, thereby making it unclear as to how Sis1p mediates propagation of [*RNQ*$^+$]. Moreover, downregulation of Sis1p or Hsp104p cures [*RNQ*$^+$] by increasing the size of Rnq1p aggregates in [*RNQ*$^+$] cells[64] beyond a certain threshold that can be effectively transmitted to daughter cells.[70] Perplexingly, the overexpression of Ssa1p also causes an increase in Rnq1p aggregate size.[71] Thus, the chaperone dynamics involved in fragmenting Rnq1p aggregates to propagate [*RNQ*$^+$] are still poorly defined.

[*RNQ*$^+$] as a Functional Amyloid: A Two-Prion System to Regulate the Formation of New Heritable Traits

Discovery of [*PIN*$^+$]. Before Rnq1p was recognized to form the [*RNQ*$^+$] prion,[25] it was discovered that the de novo formation of [*PSI*$^+$] depended on the presence of [*PIN*$^+$], a non-Mendelian factor that was presumed to be a prion.[18,19] It was later found that [*RNQ*$^+$] was the [*PIN*$^+$] element and that while Δ*rnq1* cells maintained [*PSI*$^+$], the de novo formation of [*PSI*$^+$] did not occur in Δ*rnq1* cells.[20,21] Overexpressing other Q/N-rich proteins can also confer the Pin$^+$ phenotype by enhancing the formation of [*PSI*$^+$], but [*RNQ*$^+$] does so most efficiently and does not require the overexpression of *RNQ1*.[20,72] Once [*PSI*$^+$] has formed, both [*RNQ*$^+$] and [*PSI*$^+$] can propagate independently,[19,73] and the presence of [*RNQ*$^+$] does not affect [*PSI*$^+$]-mediated nonsense suppression nor most [*PSI*$^+$]-dependent phenotypes.[19] This was the first published example of a productive interaction between heterologous prions.[74] It is interesting, then, to consider the biological consequences of [*RNQ*$^+$] and its influence on [*PSI*$^+$]. The presence of the [*PSI*$^+$] prion has profound phenotypic effects and confers growth advantages in certain conditions.[9,75,76] Not surprisingly, the reduced efficiency of translation termination can also be detrimental.[9,76] Different [*PSI*$^+$]-mediated phenotypes are observed in different genetic backgrounds.[9,76] Even different stocks of the same strain can show different phenotypes: for instance, different stocks of the strain 74-D694 show phenotypic variation and some have even lost the ability to recover from stress (refs. 9 and 76 and unpublished data). These differences highlight the complexity of many of these phenotypes and are partly due to additional mutations, but unfortunately, this has been referred to as conflicting evidence for the potential of [*PSI*$^+$] to be beneficial.[77,78] Yet, the molecular nature of some [*PSI*$^+$]-dependent phenotypes has been elucidated.[76,79] As these traits depend on the ability of [*PSI*$^+$] to act as an omnipotent nonsense suppressor, it has been proposed that [*PSI*$^+$] provides an epigenetic means for adapting to changing environments.[9-11] It is currently unclear whether [*PSI*$^+$] exists in wild strains, as the primary method used to assay for [*PSI*$^+$] in wild strains (Sup35-PFD-GFP aggregation[23]) is inconclusive as it typically requires the presence of both [*PSI*$^+$] and [*RNQ*$^+$]. For example, some [*PSI*$^+$] [*rnq*$^-$] "BSC" strains (Cox and Tuite[11]) do not show fluorescent foci when expressing Sup35-PFD-GFP

(True and Lindquist, unpublished data). Nevertheless, the existence of [*PSI*$^+$] in the wild would be predicted to be transient and may not easily survive the switch to laboratory cultivation. Since [*RNQ*$^+$] is required for [*PSI*$^+$] to form and is present in wild yeast isolates,[23,24] it follows that the [*RNQ*$^+$] prion may serve to poise cells to form [*PSI*$^+$] when environmental conditions change. In this way, this two-prion system may regulate translation and the ability of yeast to quickly adapt by using otherwise unavailable genetic information. More recently, it was discovered that [*RNQ*$^+$] is also required for the formation of [*URE3*].[22,80] [*URE3*] regulates nitrogen catabolism and may provide growth advantages in high concentrations of certain ions.[81] As such, the presence of [*RNQ*$^+$] may allow the cell to adapt to environmental changes and utilize a variety of nitrogen sources. Further examination of these interactions may eventually establish the [*RNQ*$^+$] prion as a functional amyloid.

[*RNQ*$^+$] exists in different aggregated structures. Distinct aggregated structures of the mammalian prion protein, PrP, form unique prion strains that dictate disease transmissibility and are thought to be the underlying cause of much of the variation in the pathology of prion diseases.[32] Definitive proof that changes in amyloid conformation can cause phenotypic variation came from studies of [*PSI*$^+$] prion variants. Weak [*PSI*$^+$] variants are characterized by lower levels of nonsense suppression and have aggregates that are larger, more stable, and show a slower rate of amyloid formation as compared to fibers that form strong [*PSI*$^+$] variants.[31,82-84] A model was proposed to explain the molecular basis of prion variants, positing that fiber stability and the kinetics of amyloid formation were the two primary determinants of the prion variant that propagated.[85] In contrast, for [*RNQ*$^+$] variants similarly formed from transforming in vitro fibers, only fiber stability correlates with the proposed model: aggregates of weaker [*RNQ*$^+$] variants are more stable, but exhibit a faster rate of fiber formation.[28] This suggests that the factors that determine the physical basis of [*RNQ*$^+$] variants may not be the same as those that define [*PSI*$^+$] variants.[28,85] Elucidating how different prion variants form and propagate is critical to understanding how different aggregated structures can modulate disease pathology. Indeed, even with one prion protein, different mechanisms may act to generate different classes of prion strains. Strains formed with PrP, for instance, do not all fit into one simple model that correlates biochemical and biophysical properties to in vivo propagation.[86,87]

Interestingly, the de novo appearance of [*PSI*$^+$] not only depends on the presence of [*RNQ*$^+$], but also on the prion variant of [*RNQ*$^+$]. [*RNQ*$^+$] variants were initially classified by how well they induce [*PSI*$^+$]. [*PSI*$^+$] is induced at a low frequency with the low [*RNQ*$^+$] variant, and increasing levels of [*PSI*$^+$] induction are seen with the medium, high and very high [*RNQ*$^+$] variants.[22] This classification only partly correlates to the level of aggregated Rnq1p, with decreasing levels of soluble Rnq1p seen from the low [*RNQ*$^+$] to the high [*RNQ*$^+$] variant. The very high [*RNQ*$^+$] variant was an outlier and showed the most soluble Rnq1p of the [*RNQ*$^+$] variants.[22] This change in solubility of the Rnq1 protein in these variants is often difficult to detect,[40] however, and not as marked as the changes seen with variants of [*PSI*$^+$]. Moreover, there is no correlation with the size and distribution of Rnq1p aggregates as there is for [*PSI*$^+$] variants: medium [*RNQ*$^+$] and very high [*RNQ*$^+$], for instance, exhibit nearly identical aggregate distributions.[73,82] Another means of categorizing [*RNQ*$^+$] variants is based on the aggregation pattern seen in [*RNQ*$^+$] cells expressing Rnq1p-GFP: single dot (s.d.) for a single focus of fluorescence, or multi dot (m.d.) for cells having multiple foci.[88] Curiously, the relationship between [*RNQ*$^+$] and [*URE3*] does not follow the same trend as [*RNQ*$^+$] and [*PSI*$^+$]. All of the s.d. [*RNQ*$^+$] variants induce [*URE3*] similarly, while m.d. high [*RNQ*$^+$] is unique and has the highest level of [*URE3*] formation.[22] This suggests that [*RNQ*$^+$] interacts differently with Sup35p and Ure2p to promote prion formation. Further investigation of these heterologous prion interactions will help elucidate one means by which prion variants can form de novo.

Models of [*RNQ*$^+$]-mediated [*PSI*$^+$] formation. Two predominant models have been proposed to explain how [*RNQ*$^+$] facilitates the formation of the [*PSI*$^+$] prion.[20,21] The titration model postulates that in [*rnq*$^-$] cells, some factor prevents the formation of [*PSI*$^+$], but the [*RNQ*$^+$] prion sequesters this inhibitor to allow for conversion to [*PSI*$^+$].[20,21] Alternatively, the cross-seeding model proposes that the Rnq1p aggregates in [*RNQ*$^+$] cells physically interact with Sup35p and serve as an imperfect template for [*PSI*$^+$] formation.[20,21] While these models are not mutually exclusive, no inhibitor has been found to date, even though much work has been directed toward this goal.[20] Additionally, in support of a physical interaction between Rnq1p and Sup35p, fibers of recombinant Rnq1p can seed the amyloid formation of recombinant Sup35p, albeit inefficiently, and Rnq1p and Sup35p have been shown to occasionally colocalize.[72,89] Furthermore, *rnq1* mutations have been identified that have no detectable effect on the structure of Rnq1p aggregates, but impair the formation of [*PSI*$^+$], suggesting that these residues may physically interact with Sup35p to induce [*PSI*$^+$].[43] Of course, these findings do not exclude the possibility that some cofactor is also involved in this process. Nevertheless, such cross-seeding or co-aggregation of amyloidogenic proteins may play a role in sporadic protein conformational disorders and this model provides the framework to understand how that may occur.

Non-productive amyloid interactions. In addition to facilitating prion formation, [*RNQ*$^+$] is involved in seemingly "non-productive" prion interactions, the purpose of which is unclear. For example, [*RNQ*$^+$] can induce formation of Sup35p aggregates that are not [*PSI*$^+$], termed non-heritable amyloid.[90] Unlike [*PSI*$^+$], which propagates independently after induction, the overexpression of Sup35p and continuous interaction with [*RNQ*$^+$] are required to maintain these non-heritable aggregates, which may represent by-products of [*PSI*$^+$] formation. Additionally, the presence of [*PSI*$^+$] can enhance the formation of [*RNQ*$^+$].[20]

Interestingly, [*URE3*] and [*PSI*$^+$] can antagonize each other.[91] Shortly after that discovery, it was paradoxically found that certain [*RNQ*$^+$] and [*PSI*$^+$] variants are incompatible.[88] For example, s.d. [*RNQ*$^+$] variants destabilize weak [*PSI*$^+$] by increasing the size of Sup35p aggregates.[71,88] Such destabilization was not seen for the m.d. high [*RNQ*$^+$] variant. This relationship was reciprocal for the s.d. medium [*RNQ*$^+$] and s.d. very high [*RNQ*$^+$] variants,

as the cells that did not form unstable [*PSI*$^+$] converted to [*rnq*$^-$] instead. This incompatibility was also seen with newly-induced [*PSI*$^+$]: after inducing the formation of strong [*PSI*$^+$], 70% of s.d. medium [*RNQ*$^+$] cells and 37% of s.d. very high [*RNQ*$^+$] cells became [*rnq*$^-$].[88] Furthermore, all s.d. medium [*RNQ*$^+$] cells that had stably acquired weak [*PSI*$^+$] became [*rnq*$^-$]. The mechanism behind these incompatible or non-productive prion interactions is unknown, but this may reveal how some protein aggregates can cap other aggregates or compete for cellular resources.

Mutations in *RNQ1* have also been found to negatively affect [*PSI*$^+$] propagation. Deletion of the first 100 amino acids of Rnq1p (Rnq1pΔ100) was found to inhibit the propagation of strong [*PSI*$^+$].[92] Overexpression of the Rnq1Δ100 protein eliminated both weak and strong [*PSI*$^+$] in a [*RNQ*$^+$]-dependent manner, regardless of any particular [*RNQ*$^+$] variant. Additionally, Rnq1pΔ100 inhibits [*URE3*] propagation and reduces the toxicity of polyQ aggregates.[92] Rnq1pΔ100 was later classified as a prion ([*RNQ1Δ100*$^+$]) that induces [*PSI*$^+$], but the Rnq1pΔ100-mediated induction then results in the loss of either [*RNQ1Δ100*$^+$] or [*PSI*$^+$].[93]

Most recently, 23 point mutations within the N-terminus of Rnq1p were uncovered that are phenotypically similar to Rnq1pΔ100.[42] The overexpression of these mutants resulted in the [*RNQ*$^+$]-dependent elimination of [*PSI*$^+$] by increasing the size of Sup35p aggregates.[42] Interestingly, when expressed from the native *RNQ1* promoter, these mutants did not affect [*PSI*$^+$], but impaired [*RNQ*$^+$] propagation.[41,42] Most of these mutations map to the putative α-helical domains of Rnq1p, and while the authors propose that these regions are involved in facilitating protein-protein interactions, the mechanism underlying the antagonistic prion interactions is unclear. One possibility is that disrupting the structure of the N-terminus may cause a gain-of-function effect through non-productive interactions with Sup35p that may result in capping to cure [*PSI*$^+$]. However, one can only speculate how these seemingly non-productive prion interactions relate to the potential role that [*RNQ*$^+$] may play in inducing [*PSI*$^+$] as a means of adapting to fluctuating environments. It is feasible, for example, that the incompatibility between certain protein conformers serves as a binary switch to regulate [*PSI*$^+$]-mediated adaptation.

[*RNQ*$^+$] as a Model for Pathological Amyloid

polyQ aggregation depends on [*RNQ*$^+$]. A number of inherited human diseases are caused by the expansion of glutamine repeat sequences beyond a certain threshold in particular proteins.[36] These expanded polyQ proteins are prone to aggregation that is associated with cytotoxicity and leads to neurodegeneration and ultimately death. Yeast models were created to provide a tractable means of studying the aggregation mechanisms of these proteins.[94-96] Interestingly, the overexpression of the chaperones intimately involved in prion maintenance (Sis1p, Hsp104p and Hsp70s) modulated aggregation of the huntingtin (Htt) protein that had a pathological polyQ expansion.[94] Deletion of *HSP104* effectively eliminated aggregation. The importance of these chaperones in regulating polyQ aggregation was also validated in vitro.[97] The role of these chaperones in dictating polyQ aggregation and toxicity is linked to their role in propagating the [*RNQ*$^+$] prion: both deletion of *RNQ1* and curing of [*RNQ*$^+$] suppress polyQ aggregation and its associated toxicity.[95] This also corroborated earlier findings that Pin$^+$ factors enhance the formation of polyQ aggregates.[21]

[*PSI*$^+$] also enhances polyQ toxicity and has an additive effect when [*RNQ*$^+$] is present,[96] although most yeast models of polyQ aggregation show a stricter dependence on [*RNQ*$^+$]. In a reciprocal fashion, polyQ aggregates can induce aggregation (though not prion formation) of both Rnq1p and Sup35p,[98,99] thereby showing how co-aggregation of polyQ and/or Q/N-rich proteins may play a major role in disease pathogenesis. Similarly, while sequences flanking the expanded polyQ repeats can modulate this toxicity, these sequences have the same effect when expressed in trans, and other Q-rich proteins also influence polyQ toxicity.[100,101] Importantly, however, all of these effects rely on the presence of the [*RNQ*$^+$] prion.

Insight into how Rnq1p modulates polyQ aggregation and toxicity came from the observation that mutations in endocytic proteins enhance toxicity in a [*RNQ*$^+$]-dependent manner.[102] PolyQ aggregates partially sequester the endocytic machinery and actin, thereby inhibiting endocytosis. This defect was also seen in mammalian cells.[102] It was later discovered that proteins associated with the late stages of the maturing endocytic complexes are recruited into polyQ aggregates.[103] This co-aggregation is most likely due to the polyQ stretches present in many endocytic proteins. Similarly, Rnq1p and multiple chaperones also associate with aggregates of polyQ protein in [*RNQ*$^+$] cells.[100,102,104] These protein interactions may help initiate polyQ aggregation, and the Q/N-rich Rnq1p aggregates in [*RNQ*$^+$] cells might template this process. Since Rnq1p has no known mammalian homolog, however, it is unclear whether a similar mechanism occurs in human disease. Nevertheless, the yeast model of polyQ aggregation provides a means of determining candidate proteins that may be involved in either suppressing or enhancing polyQ toxicity, and thereby provides a viable approach to identify novel therapeutic targets.[105] As such, the strict dependence of polyQ aggregation on the [*RNQ*$^+$] prion shows how [*RNQ*$^+$] can aid in studying pathological amyloid.

Rnq1p overexpression is toxic in [*RNQ*$^+$] cells. In addition to its role in facilitating polyQ toxicity, it was shown that the overexpression of Rnq1p in [*RNQ*$^+$] cells can also result in gain-of-function toxicity.[39] This toxicity required strong overexpression of full-length Rnq1p, as overexpression of either the PFD or the N-domain alone was not toxic. Overexpression of Sup35p in [*PSI*$^+$] cells can also be toxic.[33] In this case, it is clear that the essential termination complex is sequestered into [*PSI*$^+$] aggregates and the toxicity is rescued by overexpression of Sup35p's binding partner, Sup45p.[106,107] Analogously, the overexpression of Sis1p suppresses the toxicity of Rnq1p overexpression.[39] This rescue depends on the translocation of Rnq1p into the nucleus, resulting in increased Rnq1p aggregate formation.[108] Perhaps through a very different mechanism, overexpressing Rnq1p-L94A in [*RNQ*$^+$] cells is also toxic. Sis1p overexpression does not suppress the L94A-induced toxicity since this mutant impairs the Rnq1p-Sis1p interaction.[39]

Interestingly, Rnq1p-L94A overexpression is also toxic in [*rnq*$^-$] cells, which can be attributed to its ability to form "off-pathway" aggregates in the absence of [*RNQ*$^+$]. As seen for wild-type Rnq1p, however, directing Rnq1p-L94A to the nucleus via the addition of a nuclear localization signal suppresses toxicity.[108] Furthermore, nuclear Rnq1p aggregates can act in trans to sequester Rnq1p from the cytosol and repress toxicity. It has been proposed that Rnq1p overexpression causes the accumulation of an off-pathway, toxic aggregate in the cytoplasm, but the nucleus provides an environment for more efficient formation into benign aggregates. These nuclear aggregates can also localize polyQ to the nucleus.[108] Yet, instead of suppressing toxicity, nuclear translocation of polyQ enhances toxicity by decreasing the formation of SDS-resistant polyQ aggregates.[108] Hence, while the nucleus may provide a better environment for the formation of a benign amyloid structure for Rnq1p, the nuclear environment renders polyQ more soluble and more toxic.

Ydj1p has also been shown to modulate Rnq1p-associated toxicity. Overexpression of the Rnq1p-PFD is toxic in Δ*ydj1* [*RNQ*$^+$] cells, even though it is not toxic in wild-type cells.[66] In contrast to full-length Rnq1p, this toxicity is associated with the formation of SDS-resistant aggregates. The expression of Ydj1p was suggested to suppress this toxicity by binding to the Rnq1p-PFD and limiting the pool of aggregates. This suppression requires several features of Ydj1p: the zinc finger-like region (ZFLR) that is implicated in transfer of substrates to Hsp70s,[109] the C-terminal domain 1 (CTD1), which contains a hydrophobic peptide-binding pocket,[110] and farnesylation of the *CAAX* box.[66] Interestingly, these same domains were required for the Ydj1p-dependent suppression of polyQ toxicity.[66] These common mechanisms highlight the utility of Rnq1p as a model to further investigate the cellular machinery that regulates proteotoxicity. Additionally, these models may help to determine what types of protein conformers are toxic to cells.

Conclusions

While the term amyloid is generally associated with the proteins that aggregate in protein conformational disorders, there are an increasing number of examples of amyloid structures having a functional role in normal biology.[4,5] Clearly, some amyloidogenic proteins have the potential to form both toxic and non-toxic structures. From a structural standpoint, this is best detailed with the prion protein Het-s in the filamentous fungi *Podospora anserina*. Structures of Het-s associated with toxicity are amyloids having antiparallel β-sheets, whereas the benign structures identified form parallel β-sheets.[111] Similarly, the [*RNQ*$^+$] prion may simultaneously serve as an example of a functional amyloid and as a model for understanding pathological amyloid, thereby allowing us to examine a number of questions relevant to either a physiological or disease context: What is the toxic protein conformer? How are protein aggregates toxic? What types of heterotypic interactions do amyloidogenic proteins have? How can a single protein form different aggregated structures? How do these various structures cause changes in phenotype? Studying these questions using the [*RNQ*$^+$] prion will further our understanding of protein conformational disorders and perhaps also explain why evolution has preserved proteins that are susceptible to toxic conversion. Indeed, there are examples in biology of balancing selection, in which a certain trait has been conserved even though it is associated with disease, such as the sickle cell trait providing some resistance to malaria.[112,113]

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