The complexity and implications of yeast prion domains

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Prions are infectious proteins with altered conformations converted from otherwise normal host proteins. While there is only one known mammalian prion protein, PrP, a handful of prion proteins have been identified in the yeast *Saccharomyces cerevisiae*. Yeast prion proteins usually have a defined region called prion domain (PrD) essential for prion properties, which are typically rich in glutamine (Q) and asparagine (N). Despite sharing several common features, individual yeast PrDs are generally intricate and divergent in their compositional characteristics, which potentially implicates their prion phenotypes, such as prion-mediated transcriptional regulations.

The term “prions,” proteinacious infectious particles, was initially used by Prusiner to describe the causative agent of several mammalian neurodegenerative diseases known as transmissible spongiform encephalopathies.¹ It is believed that the conformational change of a host protein (PrP) is responsible for these fatal diseases.²⁻⁴ This protein-only concept was subsequently used to interpret two non-Mendelian genetic elements known as $[PSI^+]$ and $[URE3]$ in the yeast *Saccharomyces cerevisiae*, which are termed yeast prions because their transmission mechanism is similar to that of PrP^{Sc}.⁵ To date, a group of yeast prions have been identified.^{6,7} Even though it is still under debate whether yeast prions are diseases or represent evolutionary advantages, the fact that yeast prions usually do not kill cells and manifest as epigenetic traits with varying phenotypes,^{7,8} suggests that prions may have normal biological functions. Notably, yeast prion proteins usually have a definable prion domain (PrD) with diverse compositions, which is believed fundamental for amyloid formation and prionogenesis.⁹⁻¹³ In this review, the compositional features of the PrDs of several newly-identified yeast prion proteins will be compared with those of well-studied yeast prion proteins, and their phenotypic implications will be discussed.

Transcription Factors are Prevailing in the Yeast Prion Family

Currently, only two prion proteins have been reported in non-yeast organisms: the mammalian prion protein (PrP),¹ and the fungal protein HET-s in *Podospora anserina*.¹⁴ In contrast, ten prions have been identified in *S. cerevisiae* and eight of them have been assigned with a protein determinant. In fact, the history of yeast prion studies can be traced back to the 1960s, decades before Wickner proposed that the mysterious epigenetic elements, $[PSI^+]$ and $[URE3]$ were the prion forms of Sup35 and Ure2 protein, respectively.^{5,15,16} $[RNQ^+]$ (or $[PIN^+]$) is the third yeast prion identified, and is caused by the conformational changes of Rnq1.¹⁷⁻¹⁹ These three yeast prions have been extensively studied and most of our prion knowledge is based on their characteristics. Four other yeast prion proteins, Swi1, Cyc8, Mot3 and Sfp1 were recently identified as the determinants of $[SWI^+]$,²⁰ $[OCT^+]$,²¹ $[MOT3^+]$,²² and $[ISP^+]$,²³ respectively. $[NSI^+]$ is another recently discovered prion whose determinant remains to be revealed.²⁴ In addition, there are two unusual prions, $[GAR^+]$, a prion associated with heritable changes of the interaction between Pma1 and Std1;²⁵ and $[\beta]$, an infectious and active form of vacuolar protease B (PrB).²⁶

The biological function of Rnq1 is unknown and its prion form is able to dramatically enhance the $[PSI^+]$ prion conversion when Sup35 is overproduced.¹⁸ $[GAR^+]$ is involved in glucose signaling, with manifestation of resistance to glucose-repression.²⁵ The $[\beta]$ element is involved in autophagic body degradation and is essential for the proteolytic cleavage and activation of its inactive precursor protein Prb1.²⁶ Impressively, $[PSI^+]$, $[NSI^+]$ and $[ISP^+]$ can alter translation termination fidelity, whereas the other four prions ($[URE3]$, $[SWI^+]$, $[OCT^+]$ and $[MOT3^+]$) are transcriptional regulators.^{5,20-22,24,27} In addition, the $[ISP^+]$ prion protein Sfp1 is also a transcriptional factor.²³ Given the fact that five known yeast prion proteins are transcriptional regulators, transcription seems to be one of the major cellular processes affected by prion-based mechanisms.

As the number of yeast prions continuously increases, it is reasonable to speculate that there are more prion proteins existing in the *S. cerevisiae* and other organisms' proteomes. Several systematic investigations have been performed to pursue this possibility.^{22,28,29} Based on the characterized Q/N-rich PrDs of $[PSI^+]$ and $[URE3]$, Michelitsch and Weissman developed the first algorithm to screen for Q/N-rich proteins in proteomes of 31 organisms, including bacteria, budding yeast, *C. elegans* and

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Table 1. Amino acid compositions of the extended PrDs of yeast prion proteins

Protein	Ure2	Swi1	Mot3	Sfp1	Rnq1	Sup35	Cyc8	Average
position	1–89	1–385	1–295	253–331	124–405	1–135	442–678	
size(aa)	89	385	295	79	282	135	237	
Individual amino acid:								
Ala (A)	1.1	7.3	8.8	8.9	6.0	4.4	18.1	5.0
Arg (R)	4.5	1.3	0.7	1.3	1.1	1.5	0.8	4.0
Asn (N)	37.1	29.1	23.4	24.1	15.2	15.6	3.8	6.0
Asp (D)	2.2	3.1	2.4	1.3	0.0	2.2	0.4	6.0
Cys (C)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
Gln (Q)	11.2	12.7	13.6	13.9	24.8	28.9	43.0	4.0
Glu (E)	3.4	0.5	0.3	0.0	1.1	0.0	0.8	6.0
Gly (G)	5.6	2.3	2.7	1.3	17.4	15.6	2.1	5.0
His (H)	1.1	0.8	7.5	7.6	1.4	0.0	2.5	2.0
Ile (I)	3.4	2.3	3.7	5.1	0.0	0.0	2.1	6.0
Leu (L)	3.4	3.6	3.1	3.8	3.2	1.5	4.6	10.0
Lys (K)	1.1	1.6	0.3	2.5	0.4	2.2	0.4	7.0
Met (M)	2.2	1.0	2.4	5.1	2.1	1.5	1.7	2.0
Phe (F)	2.2	4.4	2.0	0.0	3.9	3.0	0.4	4.0
Pro (P)	0.0	4.7	6.8	1.3	0.7	4.4	7.6	4.0
Ser (S)	11.2	12.2	10.2	13.9	16.7	4.4	5.1	8.0
Thr (T)	5.6	9.4	5.1	8.9	0.7	0.0	3.0	6.0
Trp (W)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
Tyr (Y)	0.0	0.8	4.1	1.3	5.3	14.8	0.8	3.0
Val (V)	4.5	2.9	3.1	0.0	0.0	0.0	2.5	5.0
Combined amino acids:								
Q/N ratio	0.3	0.4	0.6	0.6	1.6	1.9	11.3	0.7
Q and N	48.3	41.8	37.0	38.0	40.0	44.5	46.8	10.0
negative charged	5.6	3.6	2.7	1.3	1.1	2.2	1.3	12.0
positive charged	5.6	2.9	1.0	3.8	1.4	3.7	1.3	11.0
hydrophobic	22.4	28.5	32.6	25.5	33.3	30.4	39.1	43.0

Definition of PrDs is based on the prediction of Alberti et al.²² Except of “Q/N ratio,” numbers in the Table represent amino acid frequencies (percentages) of PrDs analyzed by program of ProtParam.³⁶ Average amino acid percentages of yeast proteome (average) were described previously in reference 37. The green-shaded numbers represent frequencies at least 2-fold higher than the average and the pink-shaded numbers represent frequencies at least 2-fold lower than the average. Negative charged amino acids include D and E; positive charged amino acids include R and K; hydrophobic residues are A, G, C, V, P, L, I, M, F and W.

Drosophila.²⁸ They found total 107 putative Q/N-rich proteins (101 proteins with known biochemical functions or homologous to proteins of known functions) in *S. cerevisiae*. Remarkably, at least 33 (~33%) of these putative proteins are transcription factors. Later predictions using different algorithms led to similar conclusions.^{22,29} For instance, at least 25% of the 100 Q/N-rich proteins identified by Alberti et al. are transcription factors.²² Moreover, among 18 potential prion candidates identified from the 100 Q/N-rich proteins, four out of 12 proteins with known molecular functions are transcription factors (33%).²² Although the prionization of these PrD candidates needs to be further verified, these observations suggest that transcription factors are

prevalent among the putative yeast prion proteins as well. Thus, transcription may be one important cellular event that is influenced by yeast prions.

Yeast Prions Domains can be Q-, N- or Q/N-Rich

Yeast PrDs have been extensively characterized, especially for [PSI⁺], [URE3] and [RNQ⁺]. Several important characteristics of PrDs have been described based on studies of the three prions.^{9-11,30-34} Collectively, PrDs are (1) Q/N-rich; (2) intrinsically disordered; (3) modular and transferable; (4) capable of forming amyloid-based aggregates; (5) essential for prion formation

and transmission and (6) conferred by amino acid compositions, not primary sequences. In addition to Sup35, Ure2 and Rnq1, the Swi1 PrD has also been analyzed.^{12,35} Although the PrDs of Cyc8, Mot3 and Sfp1 have been predicted, they have not been systematically characterized.²¹⁻²³ Since the [β] prion is not amyloid-based and fundamentally differs from other yeast prions in the mode of inheritance,²⁶ it will be excluded from the PrD comparison henceforth. To be comparable, the location and length of each PrD herein are based on the prediction by Alberti et al.²² Amino acid compositions of the seven yeast PrDs were analyzed using program of ProtParam.³⁶

As shown in Table 1, the most striking compositional feature of the yeast PrDs is the richness of Q and N residues, with an overall content of about 37–49% in comparison to the average Q/N content of yeast proteome, ten percent.³⁷ The PrDs of Sup35 and Rnq1 have more Q than N, and their Q and N residues are dispersed rather evenly throughout the entire PrDs. In comparison, the putative Cyc8 PrD is primarily Q-rich. The predicted PrDs of Swi1, Sfp1, Mot3 and Ure2 are enriched in both Q and N, but contain more N residues, and their Q residues are seemingly not distributed evenly in their PrDs to a large extent. In particular, the Swi1 PrD is actually only attributable to a region only rich in N.¹² Thus, yeast PrDs can be N-rich, Q/N-rich or Q-rich. The significance of the Q/N residues in driving prion formation and protein aggregation has been extensively explored.³⁸⁻⁴¹ As eukaryotes contain a considerable amount of Q/N-rich proteins (107–472 per proteome) whereas prokaryotes do not,²⁸ it seems that being Q/N-rich might have been evolutionarily selected for to perform certain biological functions in eukaryotes. Although these functions are largely unknown, one such function might be to support the prion phenomenon at least in yeast.

Notably, except for Cyc8, other known transcription-relevant prion proteins are relatively N-rich. Moreover, N-rich domains are also prevalent among the predicted Q/N-rich sequences with prion activities.²² In deed, Alberti et al. found that the tested prion activities largely correlate to N richness, but not to Q richness among the 100 Q/N-rich PrD candidates.²² Thus, N might have a higher prion propensity than Q, which may explain why N-rich PrDs are prevalent for known yeast prion proteins. In a mutual substitution analysis, Halfmann et al. found that N and Q residues have opposing effects: N richness promotes assembly of amyloids, whereas Q richness promotes formation of toxic non-amyloid conformers.⁴² Molecular simulations suggest that the enhanced turn-forming propensity of N residues compared with that of Q residues might be attributable to the behavioral differences of the two amino acids.⁴² However, in another study, Q and N were found to have nearly identical prion propensities.⁴³ Virtually, the authors have pointed out that the Q content is actually positively correlated with the P content, whereas the N content is negatively correlated with the P content among the 100 Q/N-rich PrD candidates predicted by Alberti et al. which might have caused the observed differences of Q and N in prionogenesis.⁴³ Further investigation is needed to thoroughly uncover this mystery.

On the other hand, a high Q/N content might not be an absolute requirement for yeast prion formation. Considering that both PrP in mammals and HET-s in *P. anserina* are not Q/N-rich,

and many Q/N-rich proteins are not prion-prone,^{1,14,22} it seems that being Q/N-rich alone is neither necessary nor sufficient for being a functional PrD. The fact that some fragments of PrP and HET-s can act as prions in *S. cerevisiae*,^{44,45} suggests that yeast is able to support amyloid prions that are not Q/N-rich. Thus, the yeast proteome may likely have non-Q/N-rich prion proteins that need to be identified. However, identifying such PrDs is difficult due to the lack of valid prediction algorithms.

Divergent Compositional Features of the Q/N-Rich Yeast PrDs

Besides Q and N, other amino acids in a PrD may also contribute to its prion propensity.⁴⁶⁻⁴⁸ In addition to being Q/N-rich, known yeast PrDs also share several other notable compositional features.^{22,29,39,43,49} As shown in Table 1, negatively and positively charged amino acids (D, E, R and K) are under-represented in yeast PrDs. This is expected because amyloid structures disfavor charged residues.⁵⁰ Moreover, the overall content of hydrophobic residues in yeast PrDs is relatively lower than that of the average of yeast proteins (Table 1). For example, the residues C and W are completely absent in all known yeast PrDs. Hydrophobic residues have been shown to have a higher prion propensity within the context of a PrD, however, too many of hydrophobic residues might reduce the intrinsic disorder that is also essential for amyloid formation.⁴³

Other than the common characteristics listed above, yeast PrDs are generally diverse in their amino acid compositions (Table 1). For instance, S is overrepresented in the PrD of Rnq1; Y is specifically rich in the PrD of Sup35; and T is relatively enriched in the PrDs of Swi1 and Sfp1 (Table 1). Y has been shown to increase the fragmentation and heritability of polyQ proteins.⁴⁹ However, the bias toward T or Y is not observed in the PrD candidates with prion activities although S shows a modest correlation to prion activities among the 100 Q/N-rich sequences analyzed by Alberti et al.²² Moreover, G richness of the Sup35 and Rnq1 PrDs was pointed out previously and reaffirmed in this analysis (Table 1)^{46,48} and M is overrepresented in the putative PrD of Sfp1 (Table 1). However, a negative correlation was observed for M although G indeed manifests a mild positive correlation with the tested prion activities of the 100 Q/N-rich domains.²² In addition, as shown in Table 1, an unusual richness of A residues in the Cyc8 PrD is noteworthy even though A is largely inversely correlated with the tested prion activities.²² Interestingly, the overrepresented A residues in the Cyc8 PrD form more than 30 imperfect “QA” repeats.⁵¹ This alternated polar and non-polar arrangement might promote amyloid formation.⁵² Accordingly, a specific primary sequence arrangement may also affect prion propensity even though amino acid composition is considered the primary determinant of a PrD. Similar explanations may be used to understand the relative richness of P residues in the PrDs of Cyc8 and Mot3 as well as the relative richness of H residues in the PrDs of Mot3 and Sfp1 (Table 1). In general, P has a rigid structure and is a known β-sheet breaker that is disfavored by amyloidogenic proteins.^{53,54} Although amino acid H is predominantly uncharged at physiological pH, it has a very

low amyloid propensity at low pH where its side-chain is positively charged.^{55,56} Noticeably, both the P and H residues often distribute as clusters outside of the predicted PrD cores in the corresponding prion proteins. The spacing of “amyloid breaking” P residues can be critical to prion formation and the arrangement of P cluster in a PrD may be less disruptive to amyloids than the dispersed residues.^{22,47} Similarly, special arrangement may be essential for amino acid H when it is overrepresented within a PrD in order to keep the PrD functional. Similarly, oligopeptide repeats have been identified in the Sup35 and Rnq1 PrDs, but not in the PrDs of Ure2 or Swi1.^{11,46,57} Despite many reports have highlighted the importance of oligopeptide repeats in prion maintenance, recent studies suggest that the composition but not primary sequence of the repeats per se is critical for prion propagation.⁵⁸ Taken together, although the significance of the primary sequence arrangements need to be further investigated, some of these primary sequences per se may be crucial for specific PrDs in the native context.

Above observations suggest that bias of an amino acid in a PrD does not always correlate to its activity in promoting or repressing prion formation. As extended Q/N-rich regions, PrDs seem to be selected for a moderate (but not the highest) prion propensity and structural flexibility.⁴⁷ Therefore, the contents of individual amino acids in a functional PrD should be selected and balanced to satisfy these requirements in a context-dependent manner. Correspondingly, bias toward or against an amino acid in a PrD is essentially determined by the context of the PrD. For example, hydrophobic residues are more prionogenic than Q and N residues in a polar Q/N-rich context.⁴³ Considering that known yeast PrDs have a Q/N content about 37–49% (Table 1), the Q/N content outside of this range may be disfavored for prion formation. Thus, it can be inferred that Q and N might be more prionogenic than hydrophobic amino acids in a hydrophobic context.

Complex Behaviors of Yeast PrDs in the Native Context

What is the minimal size of a PrD required for prion formation and propagation? It was reported that a polypeptide with the first 61 amino acids of Sup35 can join $[PSI^+]$ aggregates in vivo and form infectious fibers in vitro.⁵⁹ Another report found that a longer fragment consisting of residues 1–83 is required to propagate a weak $[PSI^+]$ variant.⁶⁰ A recent study showed that the minimal Sup35 fragments required to form infectious fibrils in vitro span the residues 1–53, 1–40 and 1–61 when seeded by [VH], [VK] and [VL], three different variants of $[PSI^+]$, respectively.⁶¹ The minimal PrD of Ure2 was proposed to include amino acids 1–65.⁶² For Rnq1, the minimal PrD is much longer.¹¹ In the case of Swi1, it contains three obvious domains: the NH₂-terminal N-rich domain (1–323), Q-rich middle domain (337–524) and the COOH-terminal functional domain (525–1,314).¹² The N-rich domain was found to be sufficient for $[SWI^+]$ formation and propagation.¹² While the COOH-terminal domain is responsible for normal function of Swi1, the Q-rich domain is not essential for, but can modify both functions. Further truncational

analysis of the N-rich domain showed that a peptide of the first 37 amino acids is enough to decorate $[SWI^+]$ aggregates, and sufficient for $[SWI^+]$ maintenance and transmission, whereas the peptide containing the first 31 residues cannot.³⁵ These observations suggest that a yeast PrD can be as small as about 40 amino acids with a relatively simple composition. However, the minimal sizes of the PrDs have just underscored the simplicity of the yeast PrDs. More assays are necessary to fully characterize and validate the minimal PrDs described above.

In fact, a PrD can be much more complicated and its behaviors might be affected by variety factors in its native context. For instance, residues 172–289 of Rnq1 seem to be sufficient for decoration of $[RNQ^+]$ aggregates in a wild-type strain, but a longer region is required for efficient prion transmission.¹¹ Indeed, the Rnq1 PrD is a “complex” PrD including multiple Q/N-rich sub-domains, each of which can independently drive $[RNQ^+]$ formation and propagation with manifestation of species barriers.⁶³ Similarly, separate regions of the Sup35 PrD are required for efficient formation and propagation of $[PSI^+]$.⁶⁴ For other prion proteins, separate Q/N-rich regions may exist outside of the defined core PrDs. For example, Cyc8 has a second NH₂-terminal Q-rich motif (1–36, 55.6% Q) far from the Q-rich putative PrD (residues 442–678). Mot3 has an alternative N-rich region (residues 413–442) separate from the predicted PrD (residues 1–295). Similarly, Sfp1 contains three separate regions relatively rich in N residues, none of which has been tested for prion activities. These alternative Q/N-rich regions/motifs might affect the performance of the core PrDs, which remain elusive.

Furthermore, the behavior of a PrD is often affected by its flanking region(s). For instance, the flanking sequences outside of an inserted PrD that was used to replace the native Sup35 PrD significantly affected prion propagation.⁶⁵ Two very recent studies show that mutations far from the PrDs of Sup35 and Rnq1 can affect the formation and propagation of the corresponding prions,^{66,67} suggesting that PrDs may crosstalk or interact with distant regions outside of the PrDs. Interestingly, a recent study showed that coiled-coil domains are overrepresented in six known yeast prion proteins (Sup35, Rnq1, Ure2, Swi1, Cyc8 and Mot3) and in 80% of yeast prion protein candidates, which may also play important roles in prionogenesis.⁵⁸ The coiled-coil domains mainly comprise repeats of large hydrophobic residues (mainly L, I, V, M and F) with heptads spaced by other residues and can either associate, overlap or flank a PrD.⁵⁸ Their effect on PrD behaviors and prionogenesis can be varying and complex.

Implications of Novel Yeast Prion Proteins and their Structural Features

In addition to sharing some common phenotypes, individual yeast prions have unique and diverse phenotypic characteristics, especially for prions whose protein determinants are transcriptional regulators.⁷ For example, $[ISP^+]$ cannot be eliminated by deletion or overexpression of *HSP104* gene, but can be cured by guanidine hydrochloride.²³ The $[ISP^+]$ prion also does not manifest a mutant-like phenotype and forms nuclear aggregates instead of the cytoplasmic aggregates that are common for other

yeast prions. [SWI⁺] is very sensitive to alteration of Hsp70 chaperone activity and to increased temperatures.⁴⁸ In addition, several N-rich yeast prions modulating transcriptions (for example, [URE3] and [SWI⁺]) have fewer seeds and higher spontaneous rates of appearance and disappearance compared with [PSI⁺] and [RNQ⁺].⁶⁸ Since transcription factors are usually nuclear proteins and many of them have a relatively low abundance,⁴⁶ some transcription-related prions may form nuclear prion aggregates like [ISP⁺] and may have less amount of seeds, leading to unstable inheritance. More importantly, distinct compositional characteristics of PrDs may associate with distinct amyloid structures that are attributable to the different prion phenotypic features. For instance, the N-rich prions, such as [URE3], [SWI⁺], [MOT3⁺] and [ISP⁺], may have distinct amyloid structures compared with other prions relatively enriched in Q, e.g., [PSI⁺], [RNQ⁺] and [OCT⁺]. Moreover, existence of primary sequence arrangements such as oligopeptide repeats or specific amino acid clusters may also accompanied by specific amyloid structures. Due to the structural differences, amyloids can differ in accessibility to cellular factors such as molecular chaperones in charge of amyloid fragmentation, resulting different seed numbers. It has been proposed that the smaller number of seeds could account for the increased sensitivity of [SWI⁺] to chaperone alterations.⁶⁸ Possibly, a lower seed number can also be used to explain the instabilities of several transcription-linked prions.⁶⁸ Further structural assays on prion amyloid fibrils are necessary to better understand the phenotypic diversity of yeast prions, particularly the newly discovered ones.

It is surprising to see the prevalence of transcription factors among known and potential yeast prion proteins. This highlights the importance of prion-mediated mechanisms in modulating transcription and gene expression in yeast. Transcription factors

are usually organized as large protein complexes to assist in transcription, involving massive DNA-protein, and protein-protein interactions.^{69,70} Thus, a prion mechanism may be implicated in regulating global or specific sub-sets of gene expressions. How transcription is affected by prion formation in yeast? Several mechanistic models have been proposed, including titration, titration and/or modification, and antagonisms.⁷ In addition, other alternative mechanisms are also possible. For example, when a transcription factor adopts the prion form, in addition to reducing the concentration of its functional monomers caused by self-aggregation, prions may also sequester other heterologous interacting partners that might be either functionally related or unrelated. Such a phenomenon has described for Sup45, which can be sequestered by the [PSI⁺] aggregates.⁷¹ A prion may also induce the appearance of other heterogenous prion(s) and thus result in additional phenotypic changes.⁷² In addition, it is possible that even after adopting the prion conformation, some transcription factors may still be able to participate in transcriptional regulation; however, these prion proteins may have altered targets compared with their non prion isomers. In all the three cases, prion cells might manifest altered or gain-of-function phenotypes. Finally, the possibility of forming prion “strains” may provide additional fine tuning of transcriptional regulation. Further investigations will help us to elucidate how transcriptional events are influenced by prion formation.

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