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Cross-Talk between RNA and Prions

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As concepts evolve in mammalian and yeast prion biology, rather preliminary research investigating the interplay between prion and RNA processes are gaining momentum. The yeast prion [*PSI*$^+$] represents an aggregated state of the translation termination factor Sup35 resulting in the tendency of ribosomes to readthrough stop codons. This "nonsense suppression" activity is investigated for its possible physiological role to engender on *Saccharomyces cerevisiae* the ability to respond to stress or variable growth conditions and thereby act as a capacitor to evolve. The interaction between prion and RNA is a two way street—the cell may have adopted RNA processes in translation to govern the presence of prions and the [*PSI*$^+$] prion's nonsense suppressor phenotype may exhibit different growth phenotypes by its control of translation termination. RNA processes in the mammalian cell also effect and are affected by prions.

Key words: catalytic RNA, evolutionary change, IRES, nonsense suppression, prions, stress response, yeast prions.

In 1982, Stanley Prusiner coined the term "prion" to denote a small *pro*teinaceous *in*fectious particle that was distinct from nucleic acid containing viroids, viruses or plasmids based on its resistance to procedures that would normally disrupt nucleic acids (*1*). In the following 20 years, it has become widely accepted that prions are the infectious agent of a widespread number of transmissible spongiform encephalapathies (TSE) including Creutzfeldt Jakob disease (humans), bovine spongiform encephalopathy, scrapie (sheep), kuru (humans) and chronic wasting disease (deer and elk). Although now considered dogma, the protein-only prion hypothesis is nevertheless still subject to considerable debate (*2*).

A decade ago, Reed Wickner proposed the existence of yeast prions to explain the puzzling nature of [*URE3*] and [*PSI*$^+$] non-Mendelian genetic elements (*3*). During the last decade, the elucidation of many aspects of the molecular biology of these and other yeast prions have provided valuable insights into prion biology that would be inherently difficult to study in mammals. Persistent efforts to understand questions concerning prion biology including how prions are generated, how prions are controlled and the physiological meaning of prions has led us into the realm of the RNA world. Translational control, co-translational folding of prion domains and catalytic RNA all represent novel ways in which RNA processes may control the appearance and disappearance of prions in both yeast and mammals. Conversely, the yeast prion [*PSI*$^+$] has the capacity to exert remarkable control over translation termination efficiency. The purpose of this review is to outline new concepts linking prion biology to RNA.

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Saccharomyces cerevisiae translation termination factor Sup35

Of the yeast prions, the most thoroughly investigated is [*PSI*$^+$], the prion conformation of the translation termination factor Sup35 (eRF3) (Fig. 1). The translation termination activity of Sup35 is provided by the evolutionarily conserved and essential C-terminal, which interacts with Sup45 (eRF1) and has GTPase activity necessary to stimulate eRF1 in the translation termination reaction (*4*). The 123 amino acid residue N-terminal prion domain (PrD) contains 5 tandem oligopeptide repeats similar to mammalian PrP and contains a high concentration of polar glutamine and asparagine residues that are a feature of most, if not all genuine yeast prions. A highly charged middle (M) domain separates the C-terminal and N-terminal of Sup35 in *S. cerevisiae*. Although the function of this domain is unknown, the crystallographic study of an N-terminally truncated Sup35 (referred to as eRF3c) of *Saccharomyces pombe* has revealed that the positively charged M domain interacts with the specific region in the C-domain of Sup35 that serves as the Sup45-binding site (Fig. 2; Ref. *5*). Therefore, one might speculate that the M domain of Sup35 masks the Sup45-binding site and regulates Sup45 binding to Sup35 in a competitive manner. It is also intriguing to think of this M-domain/C-domain interaction as a mechanism by which the N-terminal PrD is anchored thereby inhibiting the conformational freedom it may require to adopt the [*PSI*$^+$] conformation when Sup35 is not engaged in productive interactions with Sup45. In addition the NM domain of Sup35 in *S. cerevisiae* mediates an interaction with poly-A binding protein (PABP), an interaction that is evolutionarily conserved through mammals and is thought to link translation termination with translation initiation in protein biosynthesis (*6*).

Sup35 has cellular roles apart from translation since binding partners of various cellular processes interact throughout its entire polypeptide (Fig. 1). *S. cerevisiae*

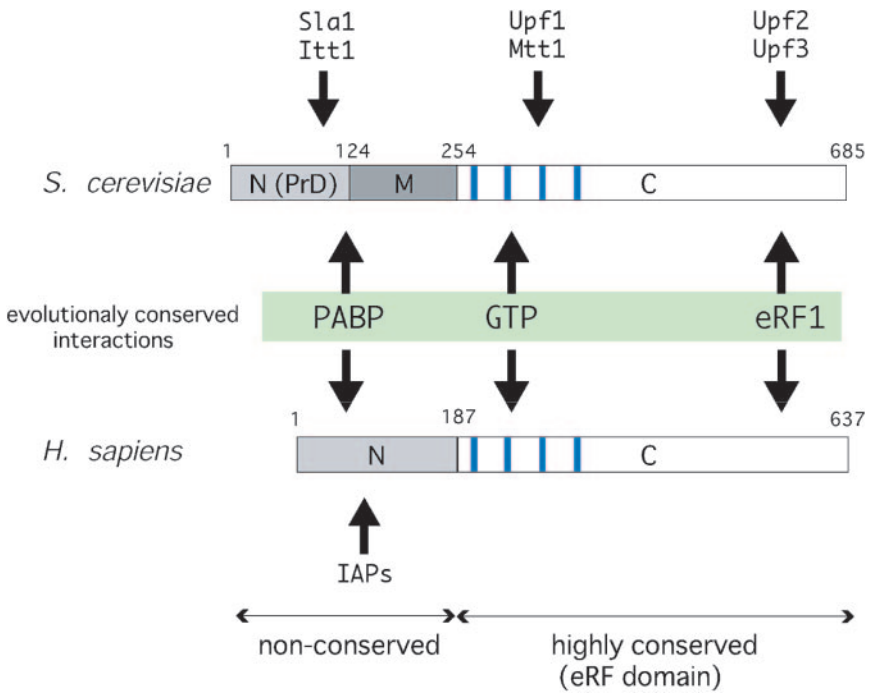

Fig. 1. **The diverse cellular roles of Sup35 in yeast and mammals as indicated by domain architecture and interacting factors in yeast and mammals.** *S. cerevisiae* Sup35 has an N-terminal 123 amino acid prion domain, followed by a charged M domain and a C-terminal domain containing 4 conserved GTPase domains (blue bars) required for translation termination. While the C-terminal is highly conserved in yeast and mammals, the N-terminal is not. Evolutionarily conserved interactions with PABP, GTP, and eRF1 are shown and are involved in translation. The N-terminal of *S. cerevisiae* also mediates interactions with Sla1 (cytoskeleton assembly) and Itt1 (function unknown) while the C-terminal mediates interactions with the Upf components of the NMD pathway, as well as Mtt1. Upf2 and Upf3 are known to compete with eRF1 for binding to Sup35. Sup35s involvement in mammalian NMD, if any, is not yet characterized. A proteolytically cleaved N-terminal fragment of mammalian Sup35 interacts with the IAP family of proteins that regulate apoptosis.

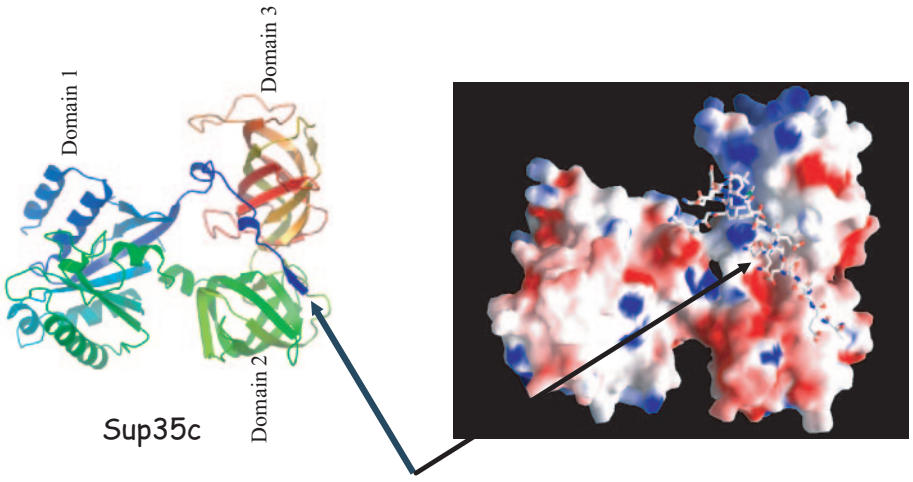

Fig. 2. **Crystal structure of N-terminally truncated Sup35 (eRF3c; Ref.** *5*) of *S. pombe* (Protein Data Bank accession codes 1R5B). (Left) The ribbon diagram of eRF3c is drawn with domains 2 and 3 in the same orientation. eRF3c contains 41 amino acids of M domain as the N-terminal extension (marked as arrows). Domains 1, 2 and 3 and the N-terminal extension of eRF3c are colored as cyan, green, orange and magenta respectively. (Right) Solvent accessible surface and electrostatic potential of eRF3c with the N-terminal extension removed for surface calculation but is shown as stick models. The region masked by the N-terminal extension overlaps the Sup45-binding site. Surface charge: positive, blue; negative, red.

Sup35 N-terminal domain also interacts with Sla1 (*7*), a component involved in the organization of the cytoskeleton. Itt1 is an additional binding partner of the N-terminal domain (*8*). Although the function of Itt1 is unknown it shares a similar RING finger motif with inhibitors of apoptosis proteins (IAPs) that interact with a proteolytically cleaved N-terminal product of eRF3 in mammalian cells (*9*). In addition, the highly conserved C-terminal of Sup35 mediates interactions with Upf1, Upf2 and Upf3, components of the nonsense mediated decay pathway (*10*) as well as the Upf1-like helicase Mtt1 (*11*).

S. cerevisiae cells lacking the N and M domains are viable, but are unable to become [*PSI*$^+$]. [*PSI*$^+$] state is caused by a conformational change in the PrD of Sup35 in which the otherwise unstructured region adopts a β-sheet rich conformation consistent with amyloid. As in PrP, this prion conformation then nucleates remaining soluble Sup35s to adopt the same conformation, leading to the growing amyloid by a mechanism termed nucleated conformational conversion (*12*). As with any inheritable element, [*PSI*$^+$] requires a replication mechanism. Otherwise, the large cytoplasmic [*PSI*$^+$] aggregate containing cells would simply be diluted out of a growing culture, and [*PSI*$^+$] cannot be heritable. This replication machinery comes in the form of heat shock protein Hsp104, which functions to break up large aggregates for resolubilization coupled with ATP hydrolysis. Hsp104 is also required for acquired thermotolerance. Hsp104 interacts with large [*PSI*$^+$] aggregates to produce seeds that are transmissible to daughter cells during cell division and is thus also required for [*PSI*$^+$] propagation (*13*). Therefore, through repeated rounds of PrD mediated aggregation and Hsp104-mediated seeding, a stably transmitted [*PSI*$^+$] phenotype is produced, marked by a reduction of soluble Sup35

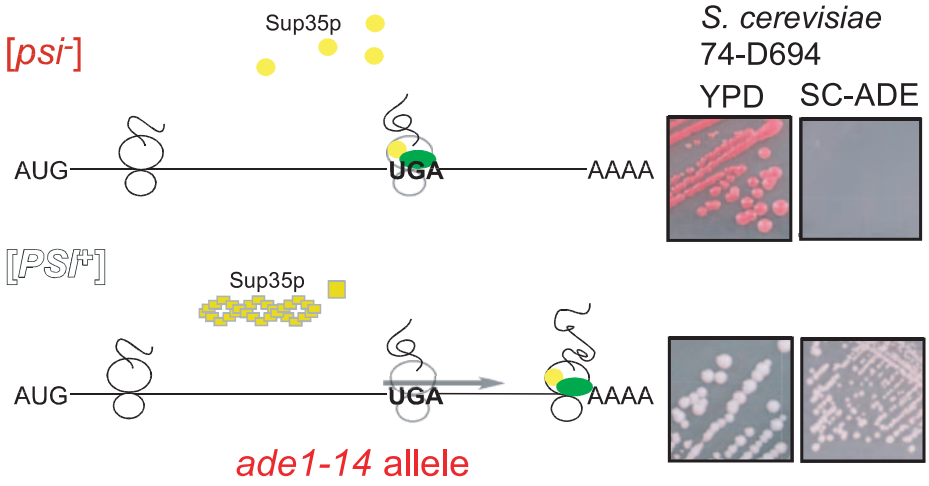

Fig. 3. **The prion form of Sup35 results in a stop codon or nonsense codon suppressor phenotype easily reported by nonsense mutation containing alleles.** In the normal [*psi*$^-$] state (above), Sup35 is in the soluble form and interacts with Sup35 to terminate translation at stop codons. In *S. cerevisiae* 74-D694, termination at the nonsense codon in *ade1-14* results in the synthesis of red pigment due to buildup of metabolic products in adenine synthesis pathway. In [*PSI*$^+$] cells (below) aggregated Sup35 results in nonsense suppression. Readthrough of the nonsense allele *ade1-14* results in cells appearing the normal white color.

and a corresponding increase in stop codon readthrough that is easily detected by nonsense suppression of certain alleles in laboratory strains of *S. cerevisiae* (Fig. 3).

However, it is of great interest whether or not "nonsense suppression" as a phenotype adopted by [*PSI*$^+$] cells, has a true physiological role. One of the more intriguing postulates is that [*PSI*$^+$] cells can take advantage of pre-existing genomic diversity to establish complex traits. Through computational survey of the *S. cerevisiae* genome, Gerstein and colleagues identified open reading frames (ORFs) of two classes that could theoretically be influenced by [*PSI*$^+$] (*14*). The first class contains ORFs that are disabled by a premature stop codon within the full protein coding sequence and the second class contains two ORFs that could potentially become a single ORF by the readthrough of a single stop codon intervening them. Many of these ORFs disabled or intervened by stop codons have properties (eg/ growth inhibition, flocculation, vanadate resistance, stress response) that are potentially related to the ability of [*PSI*$^+$] to engender substantial phenotypic variation in yeast strains under different environmental conditions. Therefore, nonsense suppression of premature stop codons within preexisting ORFs would be beneficial in diverse growth conditions.

How might [*PSI*$^+$] cells utilize such preexisting genomic variation? One might argue forthright that such genomic variation is irrelevant in the light of the nonsense mediated RNA decay (NMD) pathway. NMD functions to degrade any unwanted mRNA transcript containing a premature stop codon for avoiding build-up of possibly deleterious truncations (*15*). Importantly, normal, ie/ non-prion, Sup35 participates in the NMD pathway by interacting with components of the NMD machinery (*10*). It is known that nonsense-codon read-through (afforded by [*PSI*$^+$]), probably involving dysfunction of Sup35, antagonizes NMD in *S. cerevisiae* (*16*). Therefore, [*PSI*$^+$] provides an additional mechanism to antagonize the NMD pathway thereby allowing the exploitation of pre-existing genomic diversity in the form of ORFs with premature stop codons.

It has been previously shown that [*PSI*$^+$] cells outperformed isogenic [*psi*$^-$] cells in response to ethanol stress in three independent genotypes and in response to heat stress in two independent genotypes (*17*). In order to determine if [*PSI*$^+$] could be responsible for diverse growth phenotypes responding to a variety of external parameters, isogenic [*psi*$^-$] and [*PSI*$^+$] cells were assayed for their response to different carbon and nitrogen sources, in the presence of various inhibitors of cellular processes, stress conditions and temperature. Indeed, in a strain-dependent manner, conspicuous differences in either growth rate or colony morphology could be observed for isogenic [*PSI*$^+$] and [*psi*$^-$] cells in response to many of the growth conditions tested (*18*).

A variety of factors may influence the relative fitness of isogenic [*PSI*$^+$] and [*psi*$^-$] cells in response to various growth conditions. First, several yeast prions exist with diverse biological consequences. In fact, the presence of some prions may induce the genesis of other prions (*19*) and the various combination of prions together may exert more complex growth traits. Second, [*PSI*$^+$] prion formation is associated with aggregation. The presence or absence of such aggregates may have significant consequences for the cell (for example the expression of heat shock proteins in response to misfolded proteins) that produce distinct phenotypes in response to different growth conditions. Third, [*PSI*$^+$]'s nonsense suppression may cause the ribosome to readthrough many naturally occurring stop codons, thereby taking advantage of pre-existing genomic diversity, as outlined above. By systematically removing each of the above possibilities, it was shown that the growth phenotypes exhibited by [*PSI*$^+$] cells were a singular consequence of [*PSI*$^+$] mediated nonsense suppression. Similar [*PSI*$^+$]-like growth phenotypes could also be achieved by targeted mutations in Sup35s C-terminal that enhanced nonsense suppression independent of [*PSI*$^+$] aggregation (*20*).

Therefore, in some genetic backgrounds, the presence of [*PSI*$^+$] may provide enhanced fitness for *S. cerevisiae* to endure different growth conditions. There are several characteristics, unique to [*PSI*$^+$], that may afford upon *S. cerevisiae* a unique ability to adapt to a fluctuating environment. First, [*PSI*$^+$] is epigenetic. This allows *S. cerevisiae* to adapt to environmental conditions without the need for permanent genetic change. Since environments may fluctuate rapidly, a non-permanent response may be beneficial. Second, [*PSI*$^+$] is metastable. The *de novo* genesis and loss of the [*PSI*$^+$] phenotype occurs

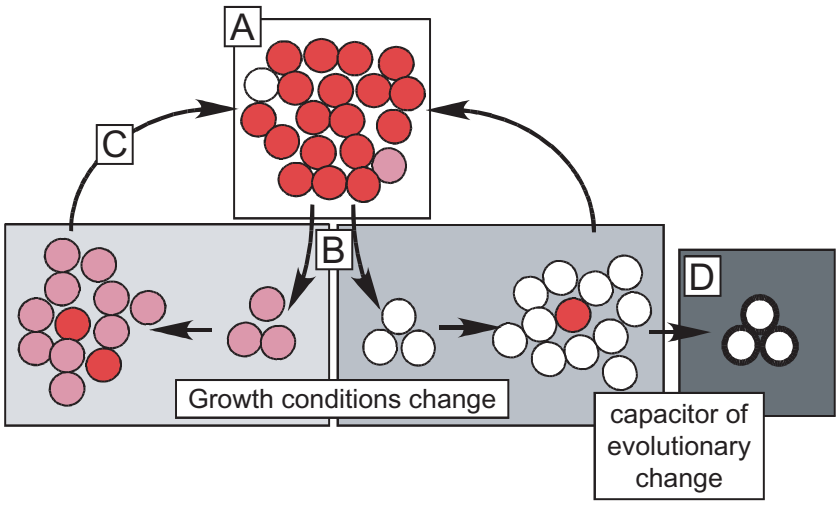

Fig. 4. **Proposed physiological role for nonsense or stop codon suppression.** [*PSI*$^+$] and [*psi*$^-$] cells of isogenic strains exhibit different growth responses to different environmental conditions. Color represents the different level of [*PSI*$^+$] state: "red," [*psi*$^-$] cells (non-prion, no read-through of *ade1-14*); "pink," weak [*PSI*$^+$] cells (weak-prion, weak read-through of *ade1-14*); "white," strong [*PSI*$^+$] cells (strong-prion, strong read-through of *ade1-14*). (A) In any population of [*psi*$^-$] cells, spontaneous conversion to different [*PSI*$^+$] strains occurs in a small proportion of cells. (B) An environmental change (grey) may select for different growth responses by weak and [*PSI*$^+$] strains. (C) [*PSI*$^+$]'s metastable nature ([*psi*$^-$] cells (red) reappear in [*PSI*$^+$] populations at low frequency) makes it ideal for returning to original environmental conditions or (D) it may provide the time necessary for genetic change to occur in response to extended environmental pressure, thereby acting as a capacitor for evolutionary change.

at low frequency, roughly 10^6–10^7 [*psi*$^-$] cells are [*PSI*$^+$]. Therefore, in any substantial population of [*psi*$^-$] cells, [*PSI*$^+$] cells will also exist with the capacity to thrive in different growth conditions, and *vice versa*. Third, the demonstrated plasticity of [*PSI*$^+$] may be ideal for responding to environments or growth conditions that fluctuate in both severity and duration. One of the more intriguing aspects of [*PSI*$^+$] is the genesis of distinct [*PSI*$^+$] strains, dependent on the tertiary or quaternary information that Sup35 adopts to become [*PSI*$^+$], within identical genetic backgrounds. Two characteristics of different [*PSI*$^+$] strains are variable levels of nonsense suppression efficiency, and mitotic stability. Therefore, it is intriguing to extend the above arguments such that any population of *S. cerevisiae* has a variable population of [*PSI*$^+$] cells with the ability to respond differently to environmental conditions. In other words, under any given environmental condition, relative fitness of strong [*PSI*$^+$], weak [*PSI*$^+$] and [*psi*$^-$] cells may ensure survival of the population (Fig. 4). Weak [*PSI*$^+$] strains, with lower nonsense suppression efficiency and readiness to return to the [*psi*$^-$] state may be more beneficial to an environmental stress that is short and mild in nature. Strong [*PSI*$^+$] cells may provide levels of nonsense suppression that allow *S. cerevisiae* to adapt to more diverse conditions over an extended period of time. The stable nature of strong [*PSI*$^+$] may even allow for [*PSI*$^+$] surviving cells to adopt the necessary genetic mutations to thrive under prolonged diverse growth conditions and this has prompted Lindquist and colleagues to describe [*PSI*$^+$] as a capacitor for evolutionary change (*18*, *20*).

Co-translational control of prion biology

Much remains to be understood about the mechanism of biogenesis of prions, ie/ when and how the structural transition from the soluble state to the prion state occurs, and this has been the focus of much study for [*PSI*$^+$]. Since the heat shock protein Hsp104 is required for [*PSI*$^+$], it was once thought that this chaperone drove the conformational conversion necessary for Sup35 to achieve the [*PSI*$^+$] state. However, Hsp104 is now thought to be involved more in the propagation of [*PSI*$^+$], and less in its biogenesis, though the role of Hsp104 in biogenesis could not be excluded at present. Another candidate is a second yeast prion, [*PIN*$^+$] (*19*) which is the prion form of yeast Rnq1 (function unknown). Only in the presence of [*PIN*$^+$], and not in [*pin*$^-$] cells, can the overexpression of the Sup35 PrD induce cells to become [*PSI*$^+$]. It is thought that [*PIN*$^+$] serves as a template for Sup35 to achieve the correct conformation for [*PSI*$^+$]. Nevertheless, [*PSI*$^+$] can also be achieved at lower frequencies by the physiological expression of *SUP35*, even in the absence of [*PIN*$^+$]. Therefore, what drives the conformational conversion of [*PSI*$^+$] under physiological conditions remains unknown.

One interesting possibility is that the information required for Sup35 to adopt the [*PSI*$^+$] state is solely determined by the N-terminal PrD and does not require the presence of other cellular factors. Rather, other cellular factors may be required to ensure that Sup35 adopts its translation-competent normal fold. Our evolving understanding of how newly synthesized proteins fold in the cytosol may support this notion. First, the information required for polypeptide folding is ejected from the ribosome in a linear manner with the N-terminal emerging before the C-terminal. For multi-domain proteins such as Sup35, polypeptides are often met at the polypeptide exit tunnel by a "welcoming committee" of ribosome-associated chaperones that eventually pass on the polypeptide to cytosolic chaperones for chaperone-assisted folding (*21*). If this chaperone network ensures the correct folding of Sup35 in [*psi*$^-$] cells, then [*PSI*$^+$] cells may result by the rare occurrence in which this system is evaded.

In yeast, ribosomal associated chaperones include the nascent chain associated complex (NAC) and Ssb. Ssb is a member of the Hsp70 chaperone family that forms a highly stable complex with the ribosome and the nascent chain emerging from the polypeptide exit tunnel (*22*). Interestingly, manipulations of Ssb activity have profound affects on [*PSI*$^+$]. Both the spontaneous formation ([*PIN*$^+$]-independent) and induction of [*PSI*$^+$] by overexpressed Sup35 ([*PIN*$^+$] dependent, Ref. *19*) are increased significantly in the absence of functional Ssb chaperone (*23*). This is a particularly intriguing finding since it is the

only example known of which the loss of a chaperone accelerates prion formation and thereby provides evidence that chaperones are required for the correct folding of Sup35 in [*psi*$^-$] cells. In addition, curing of [*PSI*$^+$] could be achieved by overexpression of Ssb (*24*) and a possible mechanism might include ensuring that Sup35 polypeptides obtain the correct fold, rather than associating and assuming the fold of Sup35 templated by [*PSI*$^+$] aggregates. Considering these findings, the biogenesis of [*PSI*$^+$] as well as other yeast prions and even mammalian PrP may be a co-translational event with the activity of factors associated with the ribosome intimately involved. One may consider the ribosome associated Ssb chaperone to be a co-translational chaperone that prevents the formation of prion aggregates. Perhaps chaperone assisted co-translational folding may be considered as a general mechanism by which not only misfolding and aggregation is avoided (*21*), but also by which the prion-fold is avoided.

The appearance or disappearance of a second yeast prion, [*URE3*] (*3*) is also regulated by a translational event that is distinct from co-translational folding. [*URE3*] is the prion form of Ure2. Ure2 binds the transcription factor Gln3 in order to block the transcription of genes involved in the assimilation of poor nitrogen sources such as arginine, urea, allantoin and ureidosuccinic acid (USA) when rich nitrogen sources are present. [*URE3*] or Δ*ura2* cells are able to use USA as a nitrogen source in the presence of ammonium sulfate, where as [*ure2-o*] cells cannot.

Ure2 is translated as a 42 kDa full-length product of 355 amino acids. A 30 kDa–truncated product, lacking the N-terminal PrD required for [*URE3*], is also produced under physiological conditions and is fully functional to regulate nitrogen synthesis. The formation of the 30 kDa–truncated product begins from a translation start codon at position 94 and is governed by an internal ribosome entry site (IRES) (*25*). A number of viral and eukaryotic mRNAs are translated by IRES in an eIF4E/Cap, eIF4G independent manner and internal initiation of eukaryotic mRNAs can occur in physiological conditions when eIF4E activity is down-regulated. The presence of an IRES element in a cellular mRNA may reflect the need to produce the corresponding protein, even when levels of translation initiation factors such as eIF4E and eIF4G are down regulated. In *S. cerevisiae*, down regulation of eIF4E resulted in an increase in the ratio of the Ure2 30 kDa product to the 42 kDa product and cells could no longer maintain [*URE3*] or transmit [*URE3*] to [*ure-o*] cells. These results indicate that accumulation of the 30 kDa product was able to cure cells of [*URE3*]. Conversely, elimination of the 30 kDa product by removal of the start codon at position 94 gave rise to high efficiency *de novo* induction of [*URE3*] (*25*). The results of this study suggest that the appearance and stability of [*URE3*] can be regulated by IRES activity governing translation of Ure2 and therefore *S. cerevisiae* has developed a specific mechanism to impede Ure2 aggregation to [*URE3*].

Mammalian prions and RNA

It is likely that additional prions and prion-like proteins will be characterized in both yeast and mammals. New evidence suggests that PrP and other prion-like proteins in mammals may govern, and be governed by RNA processes in mammals as well. Recent exciting evidence suggests that protein translation may also be regulated in mammalian cells in response to stress by a protein containing a prion-like domain. Cells subjected to environmental stresses develop cytoplasmic inclusions into which stalled translation initiation complexes are dynamically recruited termed stress granules (SGs). TIA-1 and TIAR are related proteins containing three RNA binding motifs at the N-terminal followed by a C-terminal prion-like domain that is rich in glutamine, asparagine, tyrosine and glycine residues. The PrD of TIA-1 drives the aggregation events that recruit initiating ribosomes and their associated factors that compose SGs. Similar to mammalian and yeast prions, TIA-1 induced aggregation can be induced by overexpression of the TIA-1, is resistant to proteolysis and responds to fluctuations in chaperones. Furthermore, the PrD of *S. cerevisiae* Sup35 can functionally replace the PrD-like domain of TIA-1 to drive aggregate formation required for SG synthesis (*26*).

Table 1. Comparative prion features of mammalian PrP and yeast [*PSI*$^+$]

	Mammal PrP		Yeast Sup35 [*PSI*$^+$]
Protein function	Unknown		Translation release factor
Phenotype	Neuronal cell death, human CJD, cattle BSE		Suppression of nonsense mutation
Kill organism?	yes		no
Localization	Membrane		Cytoplasm
Infectivity		yes	
Non-Mendelian inheritance		yes	
Species barrier		yes	
Prion strains		yes	
Oligo peptide repeat		yes	
Q/N rich region	no		yes
Structure		α helix → β sheet	
Protease resistance		yes	
Fiber formation *in vitro*		yes	
Aggregation *in vivo*		yes	
Prionization factor	Unknown (factor X)		Hsp104?

In mammals, as in yeast, the conditions governing the conformational conversion of PrP in the soluble form (PrP^{C}) to PrP^{Sc} is unknown. In cell-free conversion reactions, the amount of proteolysis resistant PrP material (termed PrPres) generated from a simple reaction seeded by purified PrP^{Sc} is low. However, when brain homogenates from PrP^{Sc} infected animals are used to seed the reaction, the yield of PrPres is much higher indicating that additional cellular factors may be involved in the conversion process. In addition to the usual suspects (chaperones), catalytic nucleic acids were also proposed to be such cofactors and this has been investigated in more detail, revealing that host-specific stimulatory RNA molecules aid in conversion of PrP^{C} to PrPres in cell-free conversion reactions (*2*). Comparative features between mammalian prion PrP and yeast prion [*PSI*$^{+}$] are summarized in Table 1.

Conclusions

Here, we have outlined RNA-prion interplay in both yeast and mammals as described in current research. The idea that a "nonsense suppression" phenotype may also play a physiological role in the ability of *S. cerevisiae* to cope in different growth environments represents a novel concept that if true, would have profound implications on our understanding of not only how organisms can adjust to various stresses, but evolve to meet them. Conversely, our new understanding of protein folding in the cytoplasm, co-translational folding, and translational control of translated products may provide valuable insight into the biogenesis and control of prions. Here we have outlined how RNA processes may indirectly limit prion biogenesis through monitoring folding of the polypeptide as it emerges from the ribosome exit tunnel, to more direct approaches through IRES-mediated translation to specifically inhibit the appearance of prions. It is likely that these, and additional RNA-related processes govern many other prion-related processes in yeast and mammals.

Note Added in Proof: This review referred to the following article published in Japanese: Crist *et al*. (2004) *Jikken Igaku*, 22, 2573–2581. We thank Hiroshi Kurahashi for organizing Table 1.

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