Trends in Biochemical Sciences

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Review

# Microbial Prions: Dawn of a New Era

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**Protein misfolding and aggregation are associated with human diseases and aging. However, microorganisms widely exploit the self-propagating properties of misfolded infectious protein particles, prions, as epigenetic information carriers that drive various phenotypic adaptations and encode molecular information. Microbial prion research has faced a paradigm shift in recent years, with breakthroughs that demonstrate the great functional and structural diversity of these agents. Here, we outline unorthodox examples of microbial prions in yeast and other microorganisms, focusing on their noncanonical functions. We discuss novel molecular mechanisms for the inheritance of conformationally-encoded epigenetic information and the evolutionary advantages they confer. Lastly, in light of recent advancements in the field of molecular self-assembly, we present a hypothesis regarding the existence of non-proteinaceous prion-like entities.**

## Highlights

This year marks the 55th anniversary of the discovery that prions, typically associated with human diseases, can function as information carriers in microorganisms, where they confer adaptive advantages to the cells that harbor them.

Until very recently, microbial prion research has been primarily focused on yeast, and the canonical characteristics of these epigenetic moieties included a set of typical structural and functional traits.

Recent studies have significantly expanded the definition of microbial prions; it is now clear that they are more structurally and functionally diverse than previously assumed and are widespread in the microbial world.

Microbial prions can regulate a wide variety of cellular processes and confer various evolutionary advantages by intricate molecular mechanisms, and many more paradigm-shifting examples are yet to be discovered.

## Microbial Prions: A Blessing in Disguise

Prions are self-propagating, transmittable protein particles, originally discovered in the context of neurodegenerative diseases (Box 1) [1]. It has been clear for several decades that prions are not exclusive to mammals, but rather represent a wider biochemical concept that is not necessarily associated with disease. In microorganisms, prions serve as protein-based epigenetic information carriers that confer new traits encoded in protein conformation [2]. Like their mammalian counterparts, microbial prions are unique in their self-propagation, mediated by transferring their misfolded conformation to natively folded proteins of the same kind [3] (Figure 1A).

Prionic traits are inherited in a non-Mendelian manner and can be transmitted both vertically via cytoplasmic inheritance from mother to daughter (Figure 1A) and horizontally by cell-to-cell transmission. This mechanism of protein-based inheritance can result in genome-wide changes in gene expression patterns and consequent novel microbial phenotypes, including increased stress tolerance [4], metabolic changes [5–7], cell cycle alterations [8,9], and long term memory [10,11] (Figure 2, Key Figure). Regulated coordination of these mechanisms increases the fitness of microbial communities in highly changing environments [2,4] (Figure 1B).

Microbial prions have been instrumental for understanding prions as a general biological phenomenon, as well as for unraveling the **self-assembly** (see Glossary) and propagation processes of disease-associated mammalian prions [1]. Until very recently, microbial prions have been almost exclusively described in baker's yeast (*Saccharomyces cerevisiae*) and characterized by a canonical set of structural and functional characteristics: an **amyloid** supramolecular morphology, typical prion-forming domains, and Hsp104-dependant propagation [12] (Table 1). Emerging evidence from recent years has expanded the classical concept of microbial prions, demonstrating that they are more structurally and functionally diverse and more widespread in the microbial world than previously believed.

Here, we provide a fresh overview of the current understanding of these information-carrying assemblies in light of the latest developments in the field. We showcase recent milestones in microbial prion research and discuss findings that expand the canonical notion of microbial prions

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## Box 1. Prions, Amyloids, and Human Diseases

Infection was traditionally considered to be solely conferred by living organisms or viruses. In 1976, the discovery that kuru, a disease of unknown etiology, is caused by an unidentified infectious agent which requires an extremely long incubation period and does not invoke an immune response, was accredited by the Nobel Prize in Physiology or Medicine [69]. The subsequent discovery of prions (a portmanteau of protein and infection; pronounced 'pree-on'), self-propagating proteinaceous infectious particles, made by Stanley Prusiner nearly 4 decades ago, has revolutionized the basic concepts of disease transmission and the information encoded in protein conformation, as acknowledged by the Nobel Prize in Physiology or Medicine in 1997 [70]. The notion that a simple molecular entity could induce a disease state was a true conceptual transformation that allowed us to explain puzzling conditions collectively known as 'spongiform encephalopathies', such as kuru, scrapie, Creutzfeldt–Jakob, and 'mad cow' disease [1]. The role of prions in human disease was later revealed to represent a significantly broader concept than initially appreciated. Several years ago, the intercellular prion-like transmission of protein amyloids was suggested to be the underlying mechanism for the stereotypical spreading of several amyloid-associated neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [71,72].

In most cases, prions exhibit an amyloid supramolecular morphology. Upon misfolding, most prions self-assemble and form amyloids. Thermodynamically, the self-assembly process of prions, similar to amyloids in general, is comprised of two main stages: nucleation and maturation. Nucleation is considered to be a lengthy process as the molecules move in random flow, allowing for interactions of two molecules or more. These interactions can potentiate intermolecular forces between the separate monomers and form molecular seeds, providing a template for assembly during the maturation stage (Figure I). Thus, the exogenous addition of a pre-formed seed can rapidly change the energetic state of the molecules, allowing them to bypass the nucleation stage, hence accelerating the kinetics of amyloid structure formation [73]. Amyloid fibrils typically display a cross-β morphology [74]. Aside from this nucleation–maturation mechanism, prion propagation also involves the transmission of the misfolded conformation to properly-folded monomers, hence accelerating the reaction kinetics altogether [75].

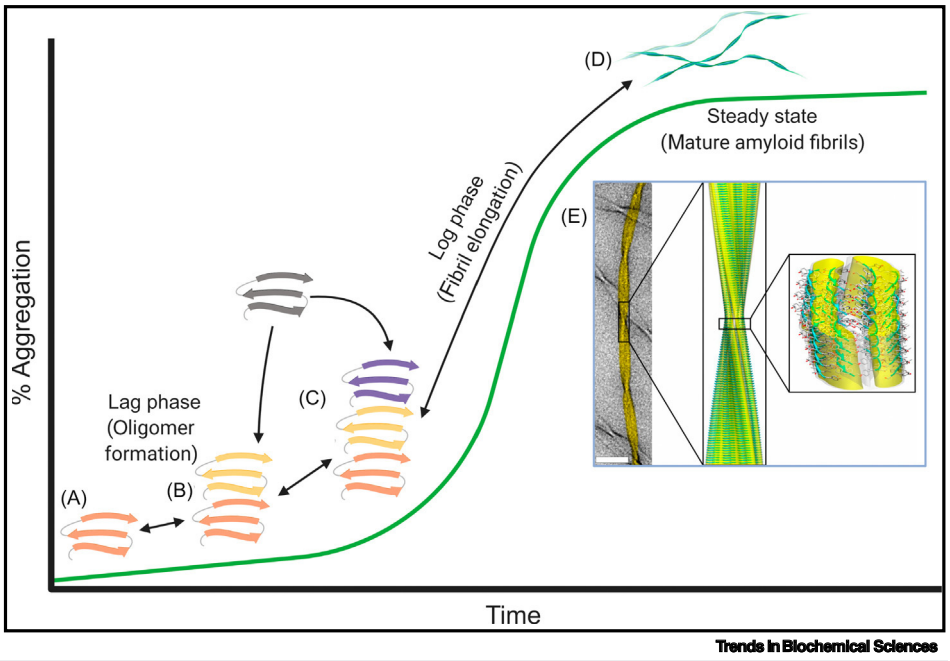



Figure I. Amyloid Assembly Kinetics. (A, B) Misfolded monomers assemble to form oligomeric nuclei (lag stage, the rate-limiting step). (C, D) In the log phase, oligomers are extended to form fibrillar amyloid fibrils (E), showing a cross-β morphology. (E) reproduced with permission from [74]. Created with BioRender.com.

## Glossary

**Amyloid:** a supramolecular nanostructure that is characterized by a set of well-defined properties, including fibrillar morphology, specific dye binding, and typical structural motifs; amyloids are classically associated with human diseases, such as Alzheimer's and Parkinson's diseases.
**Chronological lifespan:** the time a yeast cell remains alive while not dividing.
**Flocculation:** a process where yeast cells adhere to each other, forming multicellular aggregated.
**Functional amyloid:** an amyloid structure that plays physiological roles; these are especially widespread in microorganisms, where they are involved in various processes such as biofilm formation.
**Histone deacetylase:** removes acetyl groups from histones; removal of acetyl groups (hypoacetylation) transcriptionally silences the chromatin, whereas hyperacetylation activates the chromatin.
**Liquid–liquid phase separation:** the process where solutions of macromolecules (e.g., proteins or nucleic acids) form membraneless liquid droplet-like structures that are immiscible in the surrounding solution.
**Molecular chaperones:** proteins that facilitate proper folding of other proteins and/or mediate the assembly and disassembly of various macromolecular structures, such as aggregates.
**Self-assembly:** a process where molecules spontaneously arrange via noncovalent interactions into a well-defined supramolecular structure with unique functional properties.

from a structural and functional perspective, emphasizing their biological roles, evolutionary advantages, and the novel phenotypes that are conformationally encoded in these particles. Specifically, we discuss the nonclassical functions of yeast prions, such as chromatin regulation and interspecies communication. Finally, in light of recent breakthroughs in the field of molecular self-assembly, we speculate that the concept of conformationally-encoded epigenetic information might apply to much simpler molecules than previously thought.

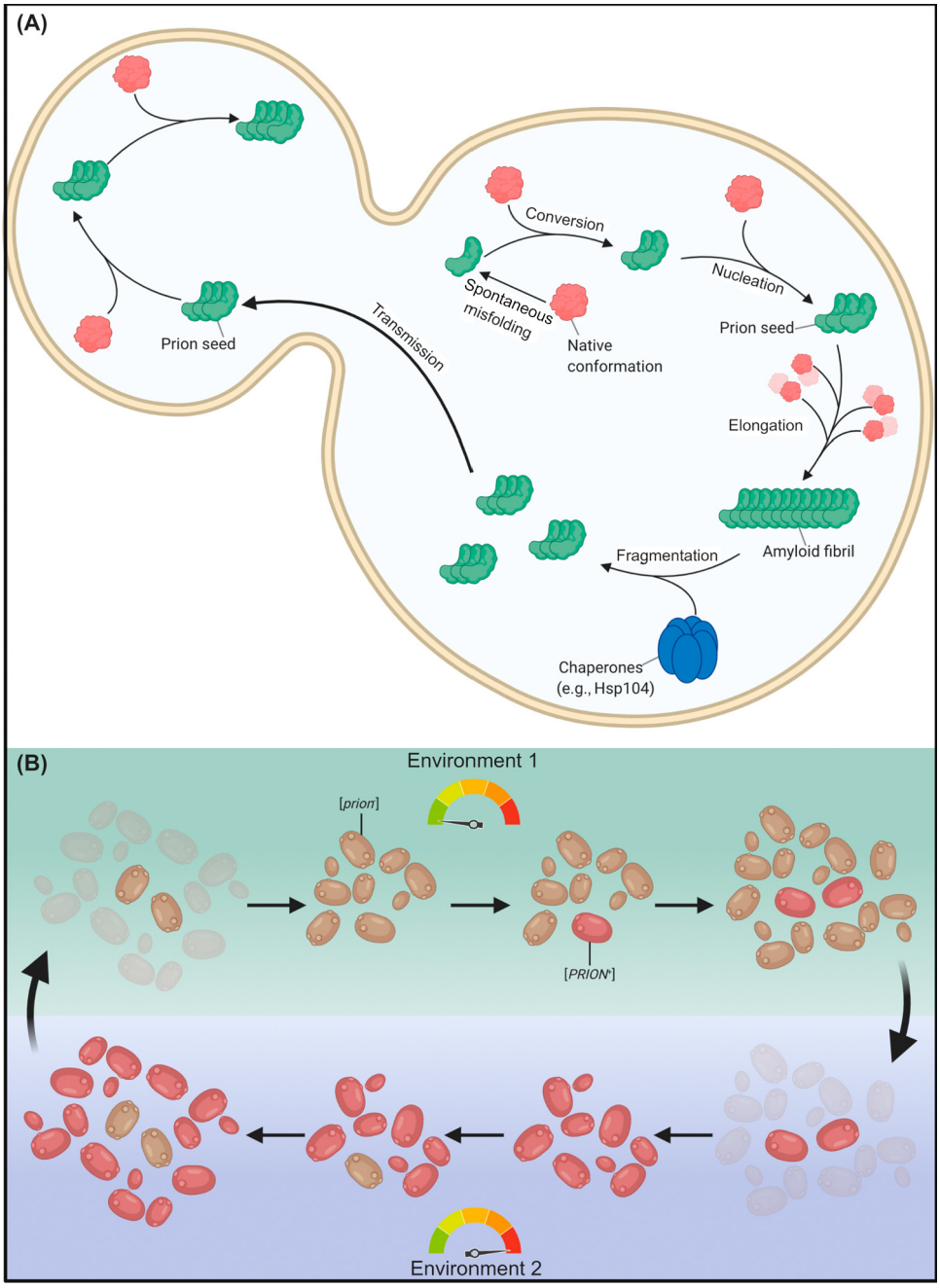
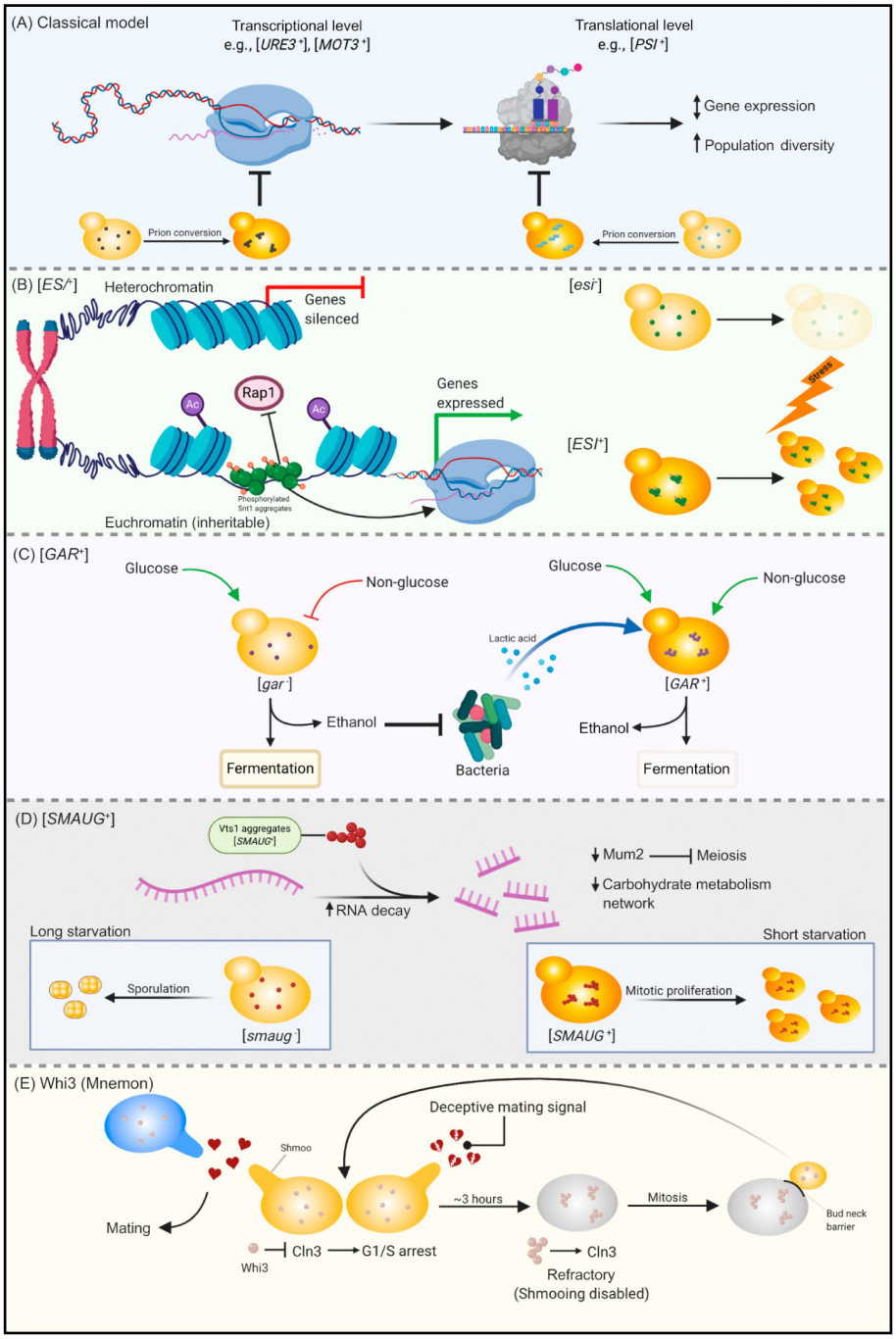



Figure 1. The Classical Model of Prion Propagation and Inheritance, and the Evolutionary Advantages of Prion Mechanisms. (A) A spontaneously formed misfolded form of a prion protein (green) can self-replicate by transforming a natively folded protein (red). Repeating events generate a prion nucleus, which is then elongated to form an amyloid fibril. Molecular chaperones fragment the fibril, generating smaller assemblies that are cytoplasmically inherited to daughter cells. Notably, it is now clear that some prions are non-amyloid, as outlined in the main text. (B) Prion-harboring cells emerge spontaneously or due to environmental signals, yet do not necessarily have an advantage in the initial environment (Environment 1). However, upon changes in the environmental conditions (Environment 2), the prion-harboring cells might be more fit and thus will thrive while the naïve cells die. Notably, unlike genetic inheritance, prion traits are reversible and can thus allow spontaneously emerging naïve cells to thrive in the previous environment. Created with BioRender.com.

## Key Figure

### Molecular Mechanisms Underlying Classical and Noncanonical Prionic Traits

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## The Amyloid State as a Prion Scaffold: Onset of Microbial Prion Research

This year marks the 55th anniversary of the first observation of prion phenotypes in microorganisms with the discovery of [*PSI+*], a suppressor of adenine auxotrophy in *S. cerevisiae* showing a non-Mendelian inheritance [13]. Three decades later, upon the maturation of the prion hypothesis in mammals, the anomalous form of inheritance of [*PSI+*] and another element designated [*URE3*] was proposed to be prionic [14]. These two elements have been extensively reviewed and studied [12,15,16], and here we only briefly outline the important points.

[*PSI+*] and [*URE3*] are the prionic forms of the Sup35 and Ure2 proteins, respectively, in *S. cerevisiae*. The soluble form of the former protein is a translation termination factor, while the latter is a transcriptional repressor of nitrogen catabolism [12,14]. Upon the transition of the soluble form into the prion conformation, the soluble proteins lose their normal function, leading to changes in gene expression patterns. In the case of [*PSI+*], loss of its translation termination function, increases the readthrough of transcriptome-wide premature termination codons (nonsense mutations), resulting in proteome-wide changes [17] (Figure 2A).

The defining hallmark of prions is their ability to infect naïve cells where they induce prion phenotypes [18–20]. The molecular mechanism underlying this mode of transmission is the capacity of prion proteins to misfold, generating oligomers that nucleate the formation of higher molecular weight species, such as amyloid fibrils. These nuclei, when transmitted to naïve cells, can initiate self-assembly (Figure 1A, Box 1). High-speed atomic force microscopy has recently provided insights into the amyloid assembly of Sup35 at a subsecond and submolecular resolution, shedding light on the complex supramolecular dynamics of the process [21].

Microbial prions can thus be viewed as a unique group of **functional amyloids** involved in information carrying [22]. Prion domains containing glutamine–asparagine-rich (QN-rich) prion domains, such as those present in Sup35 and Ure2 [18,19], have been classically considered essential for priogenicity. Yet, as outlined later, many novel prions do not contain such domains, nor form amyloids (Table 1).

The prion domain of Sup35 can drive reversible **liquid–liquid phase separation** and the formation of liquid non-amyloid condensates, that eventually solidify to form protective protein gels [23]. The phase separation is mediated by the N-terminus of the protein in response to pH changes, which are sensed via a sensor domain. Prion domain-mediated phase separation is thus a mechanism for protecting the protein from stress-induced damage [23].

Figure 2. (A) Canonical prions mainly cause phenotypic alterations due to transcriptional and translational changes, thereby regulating gene expression and increasing the population diversity. (B–E) Recent studies have revealed the remarkable complexity and diversity of prion mechanisms. (B) An epigenetic mechanism of active chromatin inheritance mediated by [*ESI*$^{+}$], the prion form of the histone deacetylase subunit Snt1. Stress-induced prolonged G2/M cell cycle arrest results in Snt1 phosphorylation, which in turn induces the prion state. The subtelomeric regions in [*ESI*$^{+}$] cells show significantly reduced binding of the transcriptional regulator Rap1, resulting in elevated expression of ~15% of the yeast open reading frames. (C) [*GAR*$^{+}$] is induced by the secretion of a diffusible chemical factor, possibly lactic acid, by bacteria that cohabitat the same environmental niches as yeast, leading to reduced fermentation. This cross-kingdom communication is beneficial to both counterparts, as it reduces the secretion of antibacterial ethanol by the yeast and allows the yeast to utilize diverse carbon sources other than glucose. (D) By elevating RNA degradation of target transcripts, the [*SMAUG*$^{+}$] prion induces mitotic proliferation under short-term starvation, thereby providing adaptive advantages in rapidly changing environments when nutrient stress is transient. (E) Whi3, an example of a Mnemon, is asymmetrically retained in the mother cell following an unsuccessful mating attempt. This noncanonical mode of inheritance maintains the meiosis inhibition of the mother cell while allowing the daughter cell to successfully mate. Created with BioRender.com.

Table 1. Prion Diversity in the Microbial World[a]

| Name of prion protein | Prion state | Organism | Protein function | Prion phenotype | Amyloid? | QN-rich domain? | Hsp104-dependant? | Refs |
|---|---|---|---|---|---|---|---|---|
| Sup35 | [*PSI*$^+$] | *Saccharomyces cerevisiae* | Translation termination factor | Genome-wide stop-codon readthrough; increased chronological life span | Yes | Yes | Yes | [17–20,37] |
| Ure2 | [*URE3*] | *S. cerevisiae* | Transcriptional repressor of genes involved in nitrogen catabolism | Loss of catabolite repression in nitrogen metabolism; allows uptake of poor nitrogen sources in the presence of ammonia | Yes | Yes | Yes | [14,94] |
| Rnq1 | [*RNQ*$^+$] / [*PIN*$^+$] | *S. cerevisiae* | Unknown | Induction of other prion proteins | Yes | Yes | Yes | [30,32] |
| Lsb2 | [*LBS*$^+$] | *S. cerevisiae* | Short-lived actin-associated protein | Triggered by thermal stress, functions like [*PIN*$^+$] | Yes | Yes | Yes | [35] |
| Mot3 | [*MOT3*$^+$] | *S. cerevisiae* | Transcription regulator of mating, carbon metabolism, and stress response | Increases stress susceptibility; induces multicellularity and flocculation | Yes | Yes | Yes | [41] |
| Mod5 | [*MOD*$^+$] | *S. cerevisiae* | tRNA isopentenyl transferase | Increased ergosterol and drug resistance | Yes | No | Yes | [42] |
| HET-s | [Het-s] | *Podospora anserina, Fusarium graminearum* | ND | Mediates heterokaryon incompatibility | Yes | No | No | [43] |
| Swi1 | [*SWI*$^+$] | *S. cerevisiae* | Subunit of the SWI/SNF chromatin remodeling complex | Poor growth on non-glucose carbon sources; reduced flocculation | Yes | Yes | Yes | [47,52] |
| Cyc8 | [*OCT*$^+$] | *S. cerevisiae* | Subunit of the Cyc8–Tup1 chromatin remodeling complex | Improved growth on non-glucose carbon sources; increased flocculation | ND | Yes | Yes | [50] |
| Snt1 | [*ESI*$^+$] | *S. cerevisiae* | Subunit of Set3C histone deacetylase | Upregulated expression of subtelomeric loci; activated chromatin inheritance | No | No | No, Hsp90 | [54] |
| Pma1–Std1 complex | [*GAR*$^+$] | *S. cerevisiae* | Plasma membrane proton pump; glucose response regulator | Utilization of non-glucose carbon sources in the presence of glucose | No | No | No, Hsp70 | [6,7,55] |
| Vts1 | [*SMAUG*$^+$] | *S. cerevisiae* | RNA binding protein | Increased proliferation under low glucose conditions; delays sporulation at short starvations | No | No | No, Hsp70 | [8,9] |
| Sky1 (Prion-like) | ND | *S. cerevisiae* | Serine-arginine kinase | Stress granule dissolution | ND | Yes | ND | [57] |
| Whi3 (Mnemon) | ND | *S. cerevisiae* | Cell cycle regulation (Cln3 cyclin inhibitor) | Memory of deceptive mating signals | No | Yes | No, but promotes assembly | [10,11] |
| Toh1 | ND | *S. cerevisiae* | Cell wall protein | Unknown; interacts with the yeast prions [*PIN*$^+$] and [PSI$^+$] | Yes | No | ND | [95] |
| Sfp1 | [*ISP*$^+$] | *S. cerevisiae* | Transcription regulator | Antisuppressor of Sup35 mutations | ND | Yes | No | [96] |
| Ctr4 | [*CTR*$^+$] | *Schizosaccharomyces pombe* | Copper transporter | Increased sensitivity to oxidative stress | ND | Yes | Yes | [97] |
| Cb-Rho | [*RHO-X-C*+] | *Clostridium botulinum* | Transcription terminator | Genome-wide changes in the transcriptome | Yes | Yes | Yes (ClpB) | [64] |
| Lef-10 | ND | *Baculoviridae* | Viral late expression factor | Regulates viral propagation upon multiple virion infection | Yes | No | Yes | [65] |

[a]Abbreviation: ND, no data.

The propagation of many prions depends on **molecular chaperones**, among which the effect of Hsp104 is best characterized. Hsp104 disaggregates large assemblies into smaller oligomeric seeds, thus initiating new rounds of self-propagation [24,25] (Figure 1A). While it plays an important role in the propagation of classical prions, like [*PSI+*] and [*URE3*], many novel prions are Hsp104-independent (yet some depend on other chaperones) (Table 1), and the molecular mechanisms that regulate their propagation are not fully understood. In a broader sense, molecular chaperones are part of a well-orchestrated network that prevents the potentially toxic effects of amyloid microbial prions [26–28].

### Heterologous Prion Interactions

Some yeast prions, such as [*PIN*$^+$] [(for [*PSI+*] inducible) or [*RNQ*$^+$]], can enhance the *de novo* formation of other prions. For example, while the soluble protein Rnq1 has no known function, the amyloid prion form is required for the formation of [*PSI*$^+$] and [*URE3*] [29,30].

Two main models for this heterologous interaction have been suggested. The first suggests a cross-seeding mechanism wherein the amyloid nuclei of [*RNQ*$^+$] physically interact with native monomers of Sup35 (or other proteins), thereby inducing the formation of misfolded nuclei [31]. The second model proposes that the effect is not specifically mediated by physical interaction between the two amyloid proteins, but rather that the interaction between preformed [*RNQ*$^+$] amyloids and the regulatory mechanisms that affect both prions alleviates the regulation of Sup35 aggregation. According to this model, [*RNQ*$^+$] titrates Hsp104 activity on Sup35, thereby promoting the assembly of the latter [32]. Indeed, the size of Sup35 seeds unexpectedly affects this interaction [33].

Notably, the interaction network between different yeast prions is more complex, as more yeast prions have been demonstrated to function like [*PIN*$^+$] [34,35]. For example, the actin-associated protein Lsb2 has recently been shown to form a metastable prion in response to thermal stress. These transient species trigger the formation of [*PSI*$^+$] prions and are subjected to regulation by the cytoskeleton and ubiquitin-dependent pathways [35].

## The Evolutionary Advantages of Prion Mechanisms

Most microbial prion-forming proteins regulate gene expression. Thus, the transition to the prion state is accompanied by robust changes at the transcriptional or translational level (Figure 2A). Unlike genetic mutations, the prion-induced altered expression patterns are transient, and thus can provide an evolutionary advantage at the population level, as they facilitate the rapid emergence of new phenotypes in highly dynamic environments [4] (Figure 1B). These phenotypes can be either advantageous or disadvantageous in the new environment. If the prion-induced phenotype increases fitness, the prion-harboring cells would proliferate although they are genotypically identical to naïve cells. The prion allows the cells to thrive in a new environment, while simultaneously maintaining their ability to succeed in the old one via spontaneous loss of the prion. Should the new environmental conditions persist, the proliferation of a fit subpopulation would increase the chances for the occurrence of permanent genetic changes that would fix the adaptive trait [4,17,36] (Figure 1B).

The mechanisms through which prions induce such changes, as well as the traits they affect, are extremely diverse. For instance, [*PSI*$^+$] enhances the phenotypic diversity and evolvability by reading through premature stop codons, thus expressing cryptic genetic information that otherwise remains silenced [4]. This results in increased tolerance to various physical and chemical stressors, such as high temperatures, ethanol, antibiotics, and other toxins [17] (Figure 2A), and also in the increased **chronological lifespan** of the yeast [37]. Despite the evidence for a beneficial contribution of [*PSI*$^+$], most variants of this prion result in deleterious phenotypes and its prion-forming ability is

not conserved in many other yeast and fungal species [38,39]. Thus, the intriguing question of whether [*PSI*$^+$] has an adaptive or detrimental value is still under debate [40].

The appearance of microbial prions can be induced by environmental stress. For instance, the transcription factor Mot3 regulates mating, carbon metabolism, and stress response. Gain and loss of [*MOT3*$^+$] prions are governed by increased ethanol concentrations and hypoxia, respectively, which interchangeably occur during yeast natural growth (i.e., the transition between fermentation and respiration). Ethanol stress triggers [*MOT3*$^+$] appearance, which induces expression of the **flocculation**-associated protein Flo11, a key determinant of multicellularity in yeast, thus inducing environment-responsive multicellular growth that increases their fitness [41] (Figure 2A). Likewise, antifungal drugs can induce the misfolding of the Mod5 tRNA isopentenyl transferase, leading to the emergence of the amyloid prion [*MOD*$^+$]. Prion emergence leads to increased ergosterol levels and elevated resistance against various fungicides [42].

Notably, while most prions affect gene expression, the HET-S/[Het-s] system in *Podospora anserina* is a unique example of a prion-based primitive 'immune system' that functions to prevent non-self-cell fusions, thus restricting viral spreading [43]. A mechanism for prion-based immunity is also found in mammals, whereupon viral infection, mitochondrial antiviral-signaling protein (MAVS) undergoes K63 polyubiquitination, resulting in its prion-like aggregation which in turn activates an innate immune response [44,45].

In the following sections, outstanding examples for novel microbial prions, their mechanisms of action, and the phenotypes they induce are outlined.

## Prions Affect Gene Expression Patterns by Regulating Chromatin State

Swi1 is part of the SWI/SNF chromatin-remodeling complex acting as a global transcription regulator, by destabilizing histone-DNA interactions, thus regulating the expression of ~6% of the yeast genome [46]. The prion form of Swi1, [*SWI*$^+$], is associated with its loss of function and amyloid formation [47,48]. The mutual function of the SWI/SNF activator–repressor complex and Cyc8–Tup1, another chromatin remodeling complex, affects gene expression [49]. Remarkably, Cyc8 can also form a prion, designated [*OCT*$^+$] [50]. The infective nature of [*OCT*$^+$] and [*SWI*$^+$] has been demonstrated, along with their dependence upon Hsp104 [47,50]. Notably, [*SWI*$^+$] is also dependent on Hsp70 and several co-chaperones [51]. [*SWI*$^+$] prion formation leads to altered expression patterns of genes which are essential for the utilization of non-glucose carbon sources [47]. Furthermore, [*SWI*$^+$] formation abolishes multicellular filamentous growth by repressing flocculin expression and other flocculation-related genes [52], while [*OCT*$^+$] exerts opposite effects [50].

While repressed chromatin state is known to be heritable via various epigenetic mechanisms, these mechanisms do not apply to activated chromatin state, suggesting it is not epigenetically inherited [53]. Most recently, this hypothesis was refuted with the discovery of an epigenetic active chromatin inheritance mechanism, via a self-templating prion formed by Snt1, a subunit of the Set3C **histone deacetylase** complex [54]. Snt1 does not harbor any typical QN-rich prion domains, and its prion conversion depends on its phosphorylation during prolonged G2/M cell cycle arrest [54]. The prion harboring cells display an elevated expression of ~15% of the yeast open reading frames, including several genes involved in metal response. Many of the upregulated transcripts are encoded by genes residing in transcriptionally repressed subtelomeric domains, designating the prion phenotype [*ESI*$^+$] for expressed subtelomeric information. The reactivated genes include homothallic mating genes (explaining the reduced mating efficiency of [*ESI*$^+$] cells), and many stress-responsive genes. Indeed, these cells show an adaptive advantage compared with naïve cells in the presence of various stressors, including metals and the antifungal

drugs fluconazole and rapamycin [54]. The [*ESI*$^+$]-mediated heritable chromatin activation is facilitated by increased acetylation, RNA polymerase II recruitment, and interference with the binding of the Rap1 telomeric repressor [54] (Figure 2B).

[*ESI*$^+$] is the first example of an environmental and cell-cycle responsive prion, that plays a role in activated chromatin state inheritance, conferring an adaptive advantage under various stress conditions [54].

## [*GAR*$^+$] Changes Metabolic Preferences in Response to Interspecies Communication

In the presence of glucose, the expression of yeast genes involved in the metabolism of other carbon sources is repressed. The [*GAR*$^+$] prion (resistance to glucose-associated repression) allows the utilization of non-glucose carbon sources in the presence of glucose [55]. [*GAR*$^+$] shows a dominant cytoplasmically-mediated inheritance and is curable by transient changes in Hsp70 chaperone levels, though its propagation is Hsp104-independent. The [*GAR*$^+$] state is regulated by glucose signaling pathway genes, and the prion structure comprises two proteins: Std1, which takes part in this pathway, and Pma1, a large P-type ATPase which plays a role in the regulation of membrane potential and cytoplasmic pH [55].

[*GAR*$^+$] is induced by the secretion of a diffusible chemical factor by diverse bacterial species, which cohabit the same environmental niches as yeast [7]. Evidence has implicated lactic acid as one of the factors mediating the bacteria–yeast communication, though other metabolites may also be involved [5]. The cross-kingdom interaction results in mutual benefits for both organisms. The induction of [*GAR*$^+$] results in the yeast producing reduced amounts of ethanol, which is toxic to the bacteria, while it allows the yeast to utilize diverse carbon sources which are often present in their natural habitat, resulting in extended lifespan [7]. This unique mechanism of cross-kingdom communication is conserved among different wild fungi [6]. Thus, by inducing a heritable epigenetic transformation, bacteria can convert their co-inhabiting yeast from metabolic specialists to metabolic generalists [6] (Figure 2C).

## [*SMAUG*$^+$] Regulates the Interplay between Proliferation and Sporulation

Yeast cells grown in poor environments can undergo meiosis, forming stress-resistant spores. However, sporulation is beneficial only if the starvation period is long; otherwise, proliferation would be favorable as it continuously increases population size. A prion-based epigenetic mechanism allows yeast cells to anticipate the duration of nutrient depletion and choose the most suitable fate based on memories made by their ancestors. This epigenetic transgenerational transfer of metabolic history, is mediated by the prion form of the RNA-binding protein Vts1, [*SMAUG*$^+$]. By promoting proliferation and repressing meiosis, [*SMAUG*$^+$] provides adaptive advantages in rapidly changing environments when nutrient stress is transient, while the [*smaug*$^-$] phenotype represses proliferation and increases sporulation, thus promoting survival under long-lasting stress [9] (Figure 2D).

Vts1 contains an intrinsically disordered region that promotes its self-assembly into gel-like condensates which propagate in a prion-like manner via a nucleation-seeding mechanism. However, unlike 'classical' prions, [*SMAUG*$^+$] assemblies are non-amyloid and depend on Hsp70 rather than Hsp104 [8]. The conversion of Vts1 into its prion form hyperactivates the protein, leading to the increased degradation rate of target RNA transcripts and robust post-transcriptional changes, including downregulation of key players in carbohydrate metabolism and storage. These changes provide a growth advantage under low glucose conditions and increase the proliferation rate [8]. Furthermore, [*SMAUG*$^+$] cells exhibit increased degradation of transcripts

encoding Mum2 (muddled in meiosis), a positive regulator of meiosis necessary for meiotic progression, thus delaying meiosis upon starvation [9] (Figure 2D).

[*SMAUG*$^+$] is widespread in laboratory strains and nature, and curing laboratory strains of the prion increased sporulation efficiency up to fourfold [9]. [*SMAUG*$^+$] variants of diverse potencies exist in different yeast populations in nature, suggesting that cells adapt the [*SMAUG*$^+$] 'strength' to the extent of environmental fluctuations they encounter [9]. Notably, the prion properties of Vts1 are also conserved in its human homolog [8].

## Sky1 Mediates Stress Granule Dissolution

Stress granules are membraneless organelles that form in the cytoplasm under various stress conditions and are dissolved when environmental conditions are reverted. The granules are composed of RNA and RNA-binding protein assemblies stalled in translation initiation [56]. Stress granule formation allows cells to spatiotemporally reorganize biochemical processes upon environmental fluctuations, thus increasing fitness [57]. Many of the RNA binding proteins in stress granules contain intrinsically disordered regions, some of which are prion-like [56] and are crucial for the recruitment of the proteins to stress granules [58]. Sky1, a serine–arginine kinase containing a QN-rich prion-like domain, plays a role in the dissolution of stress granules upon stress recovery. Sky1 is recruited to stress granules upon heat shock treatment, a process promoted by its prion-like domain. Sky1 then phosphorylates granule-residing proteins like Npl1, allowing efficient granule dissolution [57]. Notably, although Sky1 contains a prion-like domain that is essential for its recruitment to stress granules, an infective nature of the protein has not yet been demonstrated [57].

## Remember the Past, Plan for the Future: 'Mnemons'

When yeast cells are exposed to mating pheromone they arrest in the G1 phase and form cytoplasmic extensions, known as 'Shmoo', that grow towards the pheromone source. If the mating signal is deceptive and no partners are identified nearby, the cells become permanently refractory to the pheromone and resume normal proliferation. This pheromone-induced G1/S cell cycle arrest involves the translational inhibition of the G1 cyclin Cln3 by Whi3 [59]. The memory of the deceptive mating signal is epigenetically encoded via the supramolecular assembly of Whi3, leading to loss of its inhibitory effect and reactivation of mitosis [10]. Whi3 assembly is a relatively slow process (~3 hours), thereby allowing sufficient time to mate before triggering the refractory state [10] (Figure 2E). Moreover, Whi3 aggregation induces age-associated phenotypes, including sterility, that were previously incorrectly attributed to the loss of heterochromatin silencing [11].

Unlike canonical yeast prions, Whi3 assemblies are retained in the mother cell and are not inherited to daughter cells. Thus, while the mother cell is refractory and unable to shmoo, the daughter cells can successfully mate [10]. Whi3 assemblies thus allow mother cells to memorize unsuccessful mating attempts, yet these memories are not inherited to daughter cells. Due to the asymmetric nature of the Whi3 assemblies' inheritance, these were termed 'mnemons' rather than *bona fide* prions [10]. The mechanism for the asymmetric inheritance pattern probably involves the confinement of Whi3 assemblies to endoplasmic reticulum diffusion barriers formed at the bud neck, which physically restrict the assemblies to the mother cell. Defects in this barrier result in a stable prion-like mitotic propagation of the assemblies [60] (Figure 2E).

Interestingly, when Whi3 binds to target mRNA (e.g., Cln3-encoding), it can undergo liquid–liquid phase separation into distinct liquid droplets, allowing rapid spatiotemporal compartmentalization of biological processes in the absence of a membrane. This process requires its Q-rich prion-like domain [61], whereas the structure and spatial organization of Whi3-mRNA droplets depend on the secondary structure of the mRNA [62].

While more than 150 prion domain-containing proteins were identified in the yeast genome [63], the role of most remains elusive, suggesting the potential identification of additional mnemons to control the molecular history memory of the cells.

## Prions, Prions Everywhere: Bacterial and Viral Prions

For over half a century, microbial prions have been exclusively identified in fungi, especially *S. cerevisiae*. Only recently has this concept been generalized to the entire microbial world with the discovery of bacterial and viral prions.

Yuan and Hochschild have computationally identified the transcription termination factor Rho from *Clostridium botulinum* (*Cb*-Rho) as a prion [64]. When heterologously expressed and secreted by *Escherichia coli,* the 68-amino acid prion-forming domain exhibited canonical amyloid characteristics. The *Cb*-Rho prion forming domain was further demonstrated to functionally replace its counterpart in Sup35 of *S. cerevisiae.* When expressed in *E. coli*, the chimera of *Cb*-Rho and *E. coli* Rho factor aggregated *in vivo*, resulting in transcriptome-wide changes due to the effect of Rho aggregation on RNA-polymerase readthrough. Notably, the prion phenotype was cured by overexpression of the disaggregase ClpB, the bacterial analog of yeast Hsp104 [64].

The bacterial prion *Cb*-Rho and the fungal prion Sup35 are strikingly similar, as both are involved in the regulation of gene expression, which is altered upon transition to the prion conformation. While Sup35 induces changes in protein synthesis due to stop-codon readthrough in translation, prionic *Cb*-Rho induces transcriptomic changes due to transcription terminator readthrough [64]. Both expression-modifying prions provide a mechanism of increasing phenotypic variation in the population, thereby allowing microorganisms to adapt to rapidly changing environmental conditions and sustain abiotic stress factors, such as increased ethanol levels [64].

Similar to *Cb*-Rho, the LEF-10 protein encoded by the family *Baculoviridae* (viruses that infect insects), harbors a prion-forming domain that can functionally replace the prion domain in Sup35, although it is not QN-rich [65]. When insect cells are infected by multiple virions, LEF-10 undergoes aggregation and inactivation, blocking downstream viral gene expression, and thus regulating baculovirus propagation [65].

We speculate that *Cb*-Rho and LEF-10 are only representative examples of a more widespread phenomenon that is yet to be fully discovered. Indeed, a single-stranded DNA-binding protein of *Campylobacter hominis* has been recently identified as a ClpB-dependent prion [66]. These examples are not endogenous bacterial prions, as they were analyzed via heterologous expression of genes from other bacterial species in *E. coli*. Nevertheless, we anticipate that *bona fide* bacterial, archaeal, and viral prions are to be characterized in the near future.

## Concluding Remarks

Prions are a double-edged sword; in humans, they are mostly the cause of encephalopathies and are associated with neurodegenerations, while microorganisms widely exploit their self-propagating properties as epigenetic conduits of inheritance and memory. Intriguingly, a small number of beneficiary prions in higher organisms are also beginning to unveil, such as the previously mentioned MAVS adaptor protein or the Orb2/CPEB translation regulator, the prion-like filamentous aggregation of which is required for long-term memory in *Drosophila* [67,68]. Recent research has brought about novel and noncanonical examples of microbial prions that affect cell physiology by various

### Outstanding Questions

What other biological processes are mediated by prion proteins, and what are the phenotypes they induce?

How widespread are prions in bacteria, archaea, and viruses? What are the mechanisms by which they increase cell fitness?

Are there additional inheritance mechanisms of prion-like proteins?

Do prion proteins undergo phase separation *in vivo*? What are the evolutionary advantages of this process?

Do additional examples of mnemons exist? Do mnemons have functional roles in mammals?

How do different prions interact *in vivo*?

What is the full network of interspecies prion interactions?

What other supramolecular morphologies can prion-like proteins adopt?

What are the molecular mechanisms that prevent the potential toxicity of microbial prions?

How widespread and functionally diverse are beneficial prions in non-microbial organisms, including mammals?

Do the novel concepts in the field of microbial prions apply to mammals, including humans?

Do non-proteinaceous prion-like elements, like metions, exist?

unprecedented pathways (Figure 2). These newly discovered prions are distinct from classical ones, as they do not necessarily contain archetypical prion domains, can acquire non-amyloid morphologies, and are regulated by different cellular mechanisms. Furthermore, it is now clear that prions are more widespread in the microbial world than previously appreciated (Figure 3). One of the fascinating future directions is exploring the possibility that other chemical species, aside from proteins, might serve as information-encoding infective entities. We propose supramolecular structures self-assembled by various metabolites to function in a prion-like manner, thereby putatively designating them as 'metions' (Box 2). Though several findings support this hypothesis, including the potentially infective nature of amyloid-like metabolite assemblies and their cross-seeding properties, additional evidence is still required to substantiate this notion. Nevertheless, should such evidence be obtained, this concept may bear groundbreaking consequences. The rapid progress in the field raises intriguing questions that are yet to be answered (see Outstanding Questions); it is thus the dawn of a new era of microbial prion research.

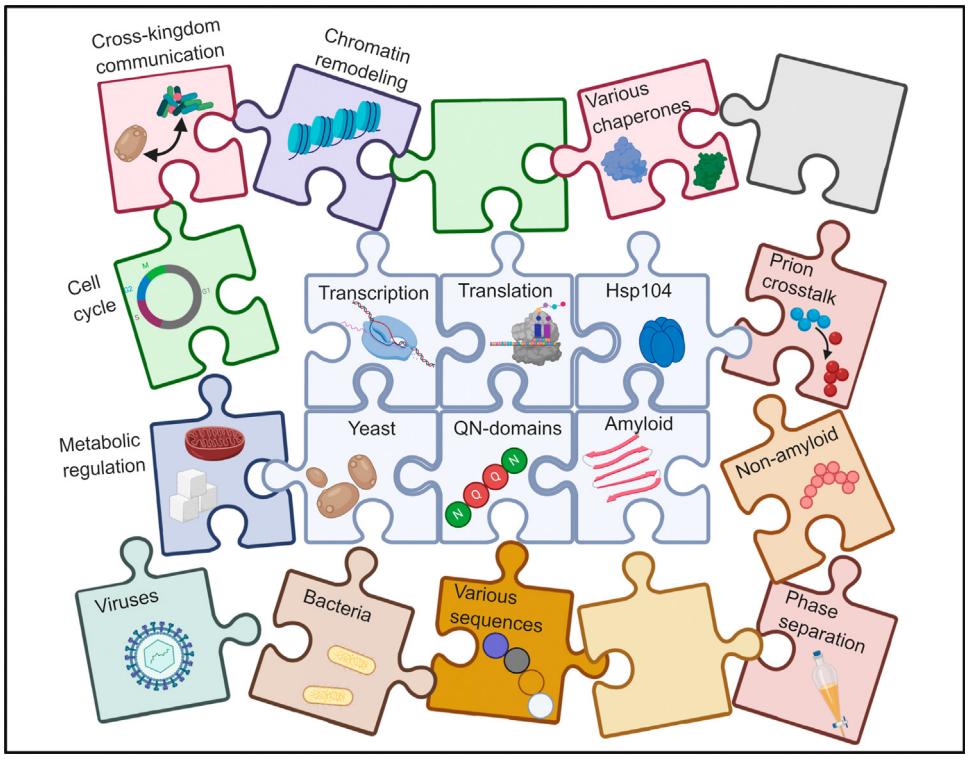


Figure 3. Microbial Prions: Dawn of a New Era. The canonical properties of microbial prions (center) have been dramatically extended in recent years from the functional and structural perspective. Initially, microbial prions were shown to function as regulators of transcription or translation, thereby modifying gene expression, and sharing common features, such as an amyloid structure, glutamine–asparagine (QN)-rich domains, and Hsp104 dependence. Yet, over the last several years, noncanonical prions have also been identified. First, in addition to yeast, prions have been found in other microbial organisms, including bacteria and viruses. Structurally, nonclassical prions do not necessarily comprise an amyloid conformation nor a QN-rich domain. Moreover, many additional functions of microbial prions have been demonstrated, including cell cycle and metabolic regulation, chromatin remodeling, and cross-kingdom communication with cohabitant bacteria. Prion crosstalk has also been reported, showing the regulation of prion formation by other prions and thus suggesting the presence of complex regulatory networks. Other interesting phenomena, such as phase separation of various prions and dependence on other chaperones, aside from Hsp104, are also being unraveled, further indicating the complexity of prion function and regulation. Thus, while significant advancements have been made lately, the pieces of this intriguing puzzle are yet to be fully assembled. Created with BioRender.com.

## Box 2. 'Metions': Do Non-proteinaceous Prions Exist?

As outlined herein, in many cases, microbial prions function to promote metabolic adaptation to changing environments by regulating gene expression patterns. This mechanism raises an intriguing question: does conformationally-encoded information represent a concept not restricted only to proteins? Specifically, can metabolite molecules directly encode and transmit metabolic information? Recent evidence has established the self-assembly properties of simple metabolites, such as amino acids and nucleobases. These can readily form supramolecular nanostructures with amyloid-like properties, including morphology, binding of specific dyes, electron diffraction pattern, intrinsic fluorescence, and membrane binding [76–79]. Amyloid-like metabolite assemblies can induce an immunological response, and exert cytotoxic effects when externally added to human cultured cells [77,80,81], indicating their internalization and potentially infective nature. A yeast model of adenine accumulation was also utilized to demonstrate the growth inhibition effect induced by intracellularly formed metabolite assemblies [82]. Moreover, retinal injection of oxalate self-assembled fibrils could recapitulate the retinopathy observed in primary hyperoxaluria patients, thus indicating a causative role for these assemblies in the disease etiology [83]. Metabolite assemblies have also been demonstrated to seed amyloid-forming proteins, like α-synuclein, thus accelerating their assembly [84]. Similarly, various metabolites have been shown to induce amyloid formation by globular proteins, as well as cross-seed other metabolites [81,85,86].

It is well known from the study of metabolite crystals that supramolecular assemblies can be formed by a nucleation-seeding mechanism [87], implying that metabolite assemblies (either amyloid or not) might form prion-like entities we term 'metions' [82]. We speculate that these metabolite infectious particles might function as prion-like elements that transmit metabolic information, mediate cell-to-cell communication, and crosstalk with the cellular proteome [88]. For instance, the metions transmitted from mother to daughter cells could be a means of intercellular communication and memory regarding the metabolic state of the cells. Such an epigenetic form of inheritance and memory regarding the cellular metabolic state could greatly increase the fitness of microbial communities.

The metions hypothesis still requires further experimental support, for instance, regarding the cell-to-cell transmission of metabolite assemblies, a key feature of prionic entities. Further validation of this notion may explain an as yet enigmatic phenomenon, concerning the maternal inheritance of human inborn errors of metabolism, wherein a recessive mutation results in a dominant disease phenotype in the heterozygous offspring of homozygous mothers [89–91]. Such an unusual mode of inheritance suggests the transmission of an infectious extranuclear agent, a characteristic of prion diseases.

Alongside metabolites, it has recently been proposed that RNAs, such as ribozymes and riboswitches, might also behave as non-proteinaceous prion-like particles that self-propagate via autocatalytic cleavage [92]. While posing a substantial challenge, revealing a prionic nature of non-proteinaceous molecules, would revolutionize basic concepts in epigenetic inheritance and transfer of chemical information by exceptionally simple elements, and bear an immense impact on our understanding of microbial physiology and human diseases (Figure I).

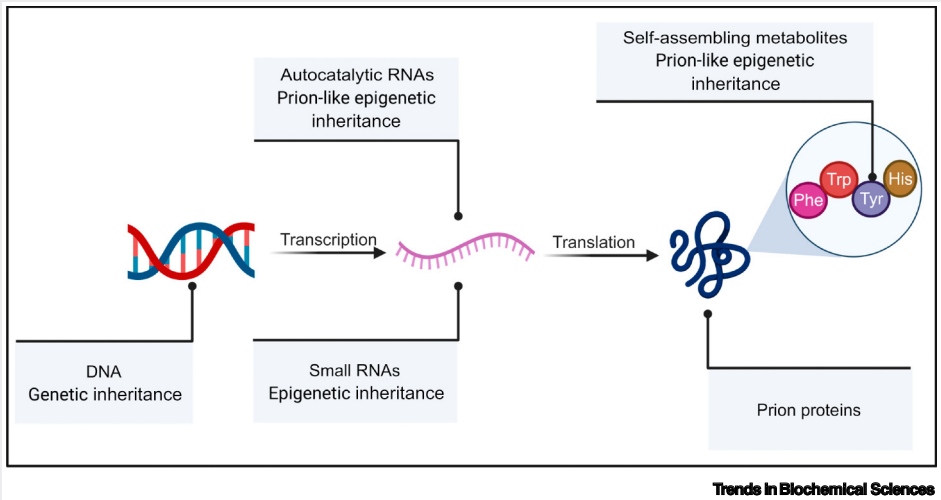


Figure I. Hypothesis: The Central Dogma in Light of the Extended Prion Concept. Alongside the classical dogma of molecular biology (DNA–RNA–Protein), epigenetic inheritance can be mediated by classical prion proteins, by prion-like RNAs [92], and possibly by metabolite assemblies (composed of amino acids, nucleobases, or others). Notably, the transgenerational epigenetic inheritance of small RNAs (non-prion) is well established [93]. Created with BioRender.com.

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