**Saccharomyces cerevisiae** in neuroscience: how unicellular organism helps to better understand prion protein?

**Abstract**

The baker’s yeast *Saccharomyces (S.) cerevisiae* is a single-celled eukaryotic model organism widely used in research on life sciences. Being a unicellular organism, *S. cerevisiae* has some evident limitations in application to neuroscience. However, yeast prions are extensively studied and they are known to share some hallmarks with mammalian prion protein or other amyloidogenic proteins found in the pathogenesis of Alzheimer’s, Parkinson’s, or Huntington’s diseases. Therefore, the yeast *S. cerevisiae* has been widely used for basic research on aggregation properties of proteins *in cellulo* and on their propagation. Recently, a yeast-based study revealed that some regions of mammalian prion protein and amyloid β_{1-42} are capable of induction and propagation of yeast prions. It is one of the examples showing that evolutionarily distant organisms share common mechanisms underlying the structural conversion of prion proteins making yeast cells a useful system for studying mammalian prion protein. *S. cerevisiae* has also been used to design novel screening systems for anti-prion compounds from chemical libraries. Yeast-based assays are cheap in maintenance and safe for the researcher, making them a very good choice to perform preliminary screening before further characterization in systems engaging mammalian cells infected with prions. In this review, not only classical red/white colony assay but also yeast-based screening assays developed during last year are discussed. Computational analysis and research carried out using yeast prions force us to expect that prions are widely present in nature. Indeed, the last few years brought us several examples indicating that the mammalian prion protein is no more peculiar protein—it seems that a better understanding of prion proteins nature-wide may aid us with the treatment of prion diseases and other amyloid-related medical conditions.

**Key Words:** amyloid; artificial prion; baker’s yeast; budding yeast; drug screening; fusion protein; neurodegenerative diseases; prion protein; yeast-based assay

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**Introduction**

Since research on Creutzfeldt-Jakob disease, kuru, scrapie, and bovine spongiform encephalopathy—today all known to be caused by proteinaceous infectious particle, or prion—has accelerated half a century ago, various experimental models have been applied to investigate the nature of infectious agent as well as its transmission pathways. Now it is known that prion is the amyloid-type aggregate of prion protein (PrP) encoded by the *PRNP* gene in mammalian genomes. Mammalian PrP is known to appear in two distinct structural forms—PrP<sup>C</sup> (cellular, monomeric) and PrP<sup>CIC</sup> (scrapie, with potential for amyloidogenesis). It is well-documented that PrP<sup>CIC</sup> amyloids are the prion particles responsible for the transmission of prion diseases including Creutzfeldt-Jakob disease in humans or scrapie among sheep (Baral et al., 2019).

Amyloidogenesis is triggered by the structural conversion of PrP<sup>C</sup> into PrP<sup>CIC</sup>. Although misfolded PrP<sup>CIC</sup> is less stable than the native PrP<sup>C</sup> conformation, its secondary structure is enriched in β-strands, which in turn can form cross-β structure across a number of PrP<sup>CIC</sup> molecules, in this way stabilizing each other. A similar phenomenon is observed for other amyloid-forming proteins that are responsible, in misfolded and aggregated forms, for the development of other neurological disorders like Alzheimer’s, Parkinson’s or Huntington’s diseases (Almeida and Brito, 2020).

As the prion diseases have been identified in mammals, experimental models utilizing mammals have been used to study i.e. inter-species transmission capabilities of these diseases. Not only primates (chimpanzees, cynomolgus macaques, or spider monkeys), but also sheep, cattle, mice, or even nematode (*Caenorhabditis elegans*) and the fly (*Drosophila melanogaster*) have been used to bring the scientific community numerous findings regarding prions and diseases caused by them (Brandner and Jaunmuktane, 2017). Recently zebrafish (*Danio rerio*) has been used as a model organism for studying the role of PrP in neuronal excitability (Kanyo et al., 2020). On the other hand, as numerous examples of iatrogenic transmission of prion diseases (Bonda et al., 2016) or transmission of bovine spongiform encephalopathy to humans causing variant Creutzfeldt-Jakob disease (Houston and Andréoletti, 2019) clearly show, research on mammalian prions in mammalian models is possibly dangerous for the...
scientific staff dealing with biological materials containing infectious prions.

The baker’s yeast *Saccharomyces (S.) cerevisiae* is a single-celled eukaryotic model organism widely used in research on life sciences. Various methods for genetic engineering of *S. cerevisiae* are commonly used, the genome is annotated very well, and the maintenance of yeast cultures is cheap in comparison to mammalian cell cultures or using animals as a model. These features of *S. cerevisiae* made this unicellular organism one of the favorite model organisms in basic research of molecular biology even serving as a living test tube for neurobiological studies. The advantages of using *S. cerevisiae*, at the same time, may bring some limitations to the research. Being a unicellular organism, *S. cerevisiae* obviously cannot serve as the best model for studying issues regarding tissues or organs. However, if basic cellular processes, i.e., protein aggregation in vivo or protein-protein interactions are studied, *S. cerevisiae* could be a good choice.

Interestingly, the *S. cerevisiae* Sup35 protein shares the hallmark of the PrP. In monomeric form Sup35p is the essential termination factor (Glover et al., 1997; Paushkin et al., 1997; King and Diaz-Avalos, 2004). It results in [PSI+] phenotype that in certain specific growth conditions gives the advantage for propagation in mammalian cells. Native PrP or Sup35 protein can be converted into a misfolded form and incorporated into the amyloids. The dotted arrows indicate hypothetical (for PrP) or non-obligatory (for Sup35p) processes, while solid arrow indicates that Hsp104p is obligatory of the propagation of the [PSI+] prion. PrP: Prion protein.

![Figure 1](https://example.com/fig1.png)

**Figure 1**  | Common characteristics of mammalian and yeast prion proteins.

Despite unrelated molecular functions of mammalian and yeast prions, they share common hallmarks. The conversion between native and misfolded structures occurs by chance and the latter one is further stabilized by aggregation. For *S. cerevisiae* it has been shown that amyloid seeds can be provided by other endogenous prions (see below). In the case of the mammalian PrP, it is not still clear, if induction and elongation of amyloids require any other factors. Both mammalian and yeast prions are self-propagating particles – amyloids can force the conversion of native protein into a misfolded form that can be incorporated into the amyloids. They are fragmented once they achieve critically large dimensions. The Hsp104 disaggregate of *S. cerevisiae* is engaged in the maintenance of this process. Such a mechanism has never been shown in the case of PrP amyloids in mammalian cells. Native PrP or Sup35 protein can be converted into a misfolded form and incorporated into the amyloids. The dotted arrows indicate hypothetical (for PrP) or non-obligatory (for Sup35p) processes, while solid arrow indicates that Hsp104p is obligatory of the propagation of the [PSI+] prion. PrP: Prion protein.

in the application of yeast cells in further studies regarding mammalian prions. Here, the most interesting application of yeast-based on the PrP study will be discussed.

One of the applications of *S. cerevisiae* in research on PrP was the expression of numerous mutant PrP proteins to map the epitope recognized by anti-PrP antibodies. By using yeast surface display method, it was shown that some antibodies fail to recognize PrP molecules due to the interaction with α-helix which is converted to β-strand upon structural conversion of whole PrP molecule (Doolan and Colby, 2015). Exact determination of epitopes for anti-prion antibodies is critical for potential immunotherapy of prion diseases. Although there are reports regarding the neurotoxicity of anti-PrP antibodies which cool down enthusiasm for immunotherapy of prion diseases (Reimann et al., 2016), this therapeutic approach is being investigated for Alzheimer’s disease by administering anti-AB and anti-tau oligomers antibodies (Vander Zanden and Chi, 2020).

There are also works bringing up the limitations of *S. cerevisiae* in studies on mammalian PrP. Jossé et al. (2012) expressed a few versions of the PrP-Sup35 fusion protein in yeast cells to study prion forming capabilities of selected fragments of PrP in *cellulo* in *S. cerevisiae*. They found that fragments originating from mammalian PrP not necessarily can ensure stable [PSI+] propagation. For example, when the PrP region known to contribute to PrP conversion to PrP[α] (amino acid residues 93–120) was fused to Sup35p instead of its native N-terminal domain, the fusion protein formed non-amyloid aggregates sensitive to sodium dodecyl sulfate (an anionic surfactant). When this fusion protein contained native Sup35 octarepeats, the aggregate was found to be sodium dodecyl sulfate-resistant, which indicates its amyloid nature, although the determination of molecular mass indicated that it was much smaller in size. Finally, the authors found that several drugs known for their anti-prion activity, including quinacrine, did not cure the prion phenotype of the yeast cells expressing PrP-Sup35 fusion proteins (Jossé et al., 2012). These results clearly show that, although safe and convenient, the yeast model might not be the best one to study mammalian PrP as it is. Rather, as it will be shown in the next section of this review, yeast prions would be the better experimental model to test drug candidates with a wide range of the amyloid targets to cure.

**Search Strategy and Selection Criteria**

The databases used to select the most relevant papers included in this article were PubMed (www.ncbi.nlm.nih.gov/pubmed/) and Google Scholar (scholar.google.com). Keywords used for the search included: mammalian prion, PRNP gene, prion protein, PrP, yeast prion, Sup35, [PSI+], anti-prion drugs, yeast-based screening system. Articles published in the period from 2000 to 2020 were analyzed, however, the original works published earlier were also considered. Additionally, articles citing papers selected as the result of the search were checked.

**Mammalian Prion Protein in *S. cerevisiae***

Formation of PrP[α] in *S. cerevisiae* cells opened a new chapter...
Recently, it has been shown that fusion protein made from Aβ1-42, the most amyloidogenic and pathogenic variant of Aβ associated with Alzheimer’s disease (Naslund et al., 1994), and N-terminal domain of Sup35p promotes the appearance of [PSI+] phenotype in yeasts. The same study covered also fusions of N fragment of Sup35p with fragments of PrP (amino acid residues 90–144, 90–152, or 90–230). Interestingly, results indicate that PrP<sub>90–152</sub> is roughly 10- and 100-fold more effective in [PSI+] induction than PrP<sub>90–144</sub> or PrP<sub>90–230</sub>, respectively (Chandramowlishwaran et al., 2018). This shows not only an interesting finding that the peptide fragment accompanied by other fragments of the same protein weakens its capabilities for nucleation, and possibly for structural conversion (PrP<sub>90–152</sub> versus PrP<sub>90–230</sub>), but also that there is a common physical mechanism underlying the amyloid formation by prion and non-PrPs between yeasts and mammals. Despite the conclusions based on the paper by Jossé et al. (2012), the recent paper by Chandramowlishwaran et al. (2018) gives some space for <i>S. cerevisiae</i> to be used as an in cellulo experimental system for studying mammalian PrP and other amyloidogenic proteins.

Limitations of yeast cells are sometimes transformed into an advantage in the design of the experiment. In mammalian cells, some mutants of PrP are known to localize in the membrane, especially those with mutations in the transmembrane segment 1 (TM1) of PrP. About half of TM1 inserted to the endoplasmic reticulum membrane has its N-terminus in the lumen of the endoplasmic reticulum (Ntm orientation), while about 20% exhibits the opposite Ctm orientation. It was shown that increased hydrophobicity of TM1 induces its Ctm orientation (Tipper et al., 2013). In this orientation, differently from the case of Ntm orientation, PrP is secreted from the cell, which in turn has a strong correlation with neuropathology in engineered mice (Hegde et al., 1998).

PrP secretion from mammalian cells requires the translocon-associated protein (TRAP) complex (Fons et al., 2003). The absence of the TRAP complex in <i>S. cerevisiae</i> causes entrapment of PrP molecules in the cell membrane even if their TM1 is inserted in Ctm orientation. By using yeast cells, it has been shown that an increase of charge difference across a transmembrane segment enhances Ctm insertion of PrP and may contribute to its neurotoxicity (Tipper et al., 2013).

**Yeast-Based Anti-Prion Drug Screening Systems**

Based on the similarities of the basic characteristics of mammalian and yeast prion proteins, <i>S. cerevisiae</i> has been exploited as an initial screening system in search of anti-prion drug candidates. If a particular chemical compound is active against yeast prions (prevents the amyloid formation or has capabilities for its disassembly) it might be active against PrP<sup>Sc</sup> amyloids. This philosophy encouraged researchers to develop yeast strains useful in a preliminary screening of libraries of chemical compounds.

The most widely recognized system is based on [PSI+] strain with ade1-14 or ade2-1 mutation in ADE1 or ADE2 genes, respectively, which namely are premature termination codons (PTCs) in genes essential for adenine biosynthesis (Ishikawa, 2008). Unless [PSI+] is cured (i.e., amyloids are disassembled to monomeric Sup35p molecules) to become [psi−], ade1-14 and ade2-1 strains readthrough PTCs and produce active Ade1 and Ade2 enzymes that catalyze coenzyme biochemical reactions. However, once ade1-14 or ade2-1 cells become [psi−], they begin to properly recognize PTCs and fail to produce active enzymes. In low adenine conditions, these cells start to accumulate intermediate products of adenine biosynthesis (phosphoribosylaminomimidazole and/ or phosphoribosylaminomimidazole carboxylate), which after oxidation or hydroxylation by cytochrome P450 enzymes form conjugates with reduced glutathione by a non-enzymatic manner. Finally, these molecules are transported to vacuoles to form red pigment, which makes colonies of cured cells red (Figure 2A).

Applications of this test in search of anti-prion drug candidates will be discussed below, but it is worth to mention here that the requirement of a sufficient level of reduced glutathione to develop red pigment has been used for another amyloid-related yeast-based assay to evaluate oxidative stress caused by overexpression of amyloidogenic proteins. The appearance of white colonies upon overexpression of these proteins and reversions to red ones after shutting off the transcription confirmed that amyloids, like Aβ<sub>1-42</sub>, expressed in <i>S. cerevisiae</i> generate oxidative stress (Bharathi et al., 2016). Interestingly, exposure of the yeast cells to 2.0 GHz radiofrequency electromagnetic field, the same frequency as used in the 4th generation long-term evolution and the 5th generation wireless technologies for digital cellular networks, caused the de novo formation of yeast prions, as well as resulted in the elevated level of reactive oxygen species and expression of superoxide dismutase and catalase (Lian et al., 2018). These yeast-based findings might be a preceding result, which should be investigated also concerning amyloids found in mammalian neurons.

Screening of anti-prion drug candidates utilizing ade1-14 or ade2-1 strains are performed as follows: ade1-14 [PSI+] or ade2-1 [PSI+] strain is spread on the surface of the solid growth medium, small disks made from filter paper are placed on the surface and chemical compounds to be assessed are spotted on the disks. After a few days of growth, the surface of the growth medium is covered with numerous white colonies, whereas around the disks containing active drugs against [PSI+] prion colonies are red (Figure 2A). In case the drug candidate is lethal for yeasts, there is a clear zone on the surface of the growth medium around the disk.

This screening method has been utilized for decades to reveal several anti-prion drug candidates. Doxorubicin (Tagliavini et al., 1997), quinacrine (Doh-Ura et al., 2000; Korth et al., 2001), kastellpaolitines, 6-aminophenanthridine (Bach et al., 2003), pentosan polysulfate (Rainov et al., 2007), guanabenz acetate (Tribouillard-Tanvier et al., 2008), imiquimod (Oumata et al., 2013), and bromotyrosine derivatives (Jennings et al., 2018) have been positively evaluated by red/white colony assay. Some of these compounds have been further evaluated by different experimental approaches. On the prion-infected mouse Scn2A neuroblastoma model, it has been shown that pentosan polysulfate directly binds to PrP<sup>Sc</sup> and at the same time decreases PrP<sup>Sc</sup> level, although its mechanism has not been determined (Yamasaki et al., 2014).

Besides mammalian neurons infected by prions widely used as a model system, one of the emerging anti-prion assays is a shaking-induced conversion of PrP. Shaking of the solution containing recombinant PrP boosts its aggregation, which can be easily and quantitatively evaluated by resolution enhanced native acidic gel electrophoresis (Ladner-Keay et al., 2018). This study revealed that quinacrine binds specifically to PrP<sup>Sc</sup> and prevents this protein from forming amyloid fibrils (Ladner-Keay et al., 2018). The shaking-induced conversion method shares some principles with a real-time quaking-induced conversion method described earlier (Atarashi et al., 2011). Differently from the shaking-induced conversion method, real-time quaking-induced conversion is quantified by the measurement of fluorescence emitted due to specific interaction of thiouvin T with PrP<sup>Sc</sup> amyloids (Green, 2019).

It should be stressed that the red/white colony assay has been further improved for high-throughput studies. Jennings and colleagues recently showed that butenolides and diphenylpropanones isolated from ascidian tunicate
Polycarpa procera have anti-prion activity by the liquid version of red/white colony assay. They reported that the assay was performed in 96-well or 384-well plates containing yeast mini-cultures supplemented with investigated chemical compounds. Next, plates were photographed and red color intensity was measured by image analysis software or quantified by measuring fluorescence (excitation 544 nm/emission 620 nm) on a microplate fluorimeter (Jennings et al., 2019).

A similar approach but the different mechanism has been employed in recently published high-throughput anti-prion activity assay. Briefly, the novel assay is all about the ability to grow on ura− medium, while when cured they produce yeast cells to grow on liquid medium lacking uracil−when cured. It indicates that even in a preliminary screening of libraries of chemical compounds performed on yeast-based systems, false-positive or false-negative results may arise. Current availability of at least three different yeast-based screening assays ([PSI]+ red/white assay, [SWI]+ growth assay, and [LEU2]+ growth assay) makes it possible to cross-check preliminary results on cheap and safe yeast-based assays, to proceed to mammalian cell-based tests with only well-evaluated chemical compounds.

Prions are more common than once thought to be?

For a long time, the [PSI]+ prion was the representative yeast prion together with other few well-studied examples including [URE3] and [PIN]+. It seemed, more or less, like yeast...
PrPs are rather uncommon and awkward protein examples just as PrP in mammalian cells was. This landscape radically changed in 2009 when a breakthrough paper by Alberti et al. (2009) was published. By analyzing the S. cerevisiae genome, they found about 200 putative PrDs and confirmed 19 of these to form prion in vivo (Alberti et al., 2009). Among newly discovered prions, there was [MOT3'], which is an aggregated Mot3 transcriptional regulator. It can be easily imagined how pleiotropic the effects of such aggregation and disaggregation might be. From this moment, many yeast prions have been discovered. Among biologically interesting examples the [GAR'] prion should be mentioned. This prion phenotype cancels catabolic repression and facilitates yeast cells to catabolize non-fermentative carbon sources even in the presence of glucose (Jarosz et al., 2014). Follow-up studies revealed that wild-type yeast cells can become [GAR+] and [SMAUG+] prion phenotype. It should be noted that the [GAR+] prion phenotype is determined by [VTS1] prion, or aggregated Vts1p, appears to be a non-amyloid, self-assembling, gel-like particle that hyperactivates its component (Chakravartty et al., 2020). The Vts1 protein binds specific hairpin structures.

The [SMAUG+] prion, or aggregated Vts1p, appears to be a non-amyloid, self-assembling, gel-like particle that hyperactivates its component (Chakravartty et al., 2020). The Vts1 protein binds specific hairpin structures is the advantage for bacteria residing in the same ecological niche as yeasts, because reduced production of ethanol by yeast cells may favor bacterial growth.

### Table 1

| Native protein | Molecular function | Prion phenotype | References |
|---------------|-------------------|-----------------|------------|
| Sup35         | Translation termination factor eRF3 | [PS'] | Translational read-through | Cox, 1965 |
| Ure2          | Nitrogen catabolite repression transcriptional regulator | [URE3] | Utilization of poor nitrogen sources | Ishikawa, 2008; Cox and Tuite, 2018; Manjrekar and Shah, 2020 |
| Rnq1          | Unknown | [PIN'] | Facilitation of the de novo appearance of other prion phenotypes | Lacroute, 1971 |
| Prb1          | Vacular proteinase B with H3 N-terminal endopeptidase activity | [B] | Increased viability during starvation; necessary for sporulation | Chen et al., 2011; Manjrekar and Shah, 2020 |
| Swi1          | Subunit of the SWI/SNF chromatin remodeling complex | [SWT'] | Altered carbon source utilization | Derkatch et al., 1997; Serio, 2018; Manjrekar and Shah, 2020 |
| Mot3          | Transcriptional repressor and activator | [MOT3'] | Altered cell wall composition | Alberti et al., 2009 |
| Cyc8          | General transcriptional co-repressor | [OCT'] | Higher levels of invertase activity under glucose-repressed conditions; increased flocculence | Crow and Li, 2011; Chernova et al., 2014; Manjrekar and Shah, 2020 |
| Sfp1          | Transcriptional regulator of ribosomal protein and biogenesis genes | [ISP'] | Suppression of nonsense codon read-through | Pat et al., 2009 |
| Pma1 and Std1| Plasma membrane P2-type H+ - ATPase that pumps protons out of cell; Regulator interacting with kinase Snf1p involved in control of glucose-regulated gene expression | [GAR'] | Breakdown of a wide range of carbon sources in the presence of glucose | Crow and Li, 2011; Chernova et al., 2014; Manjrekar and Shah, 2020 |
| ModS          | Δ'-isopentenyl pyrophosphate:tRNA isopentenyl transferase required for biosynthesis of isopentenyladenosine in mitochondrial and cytoplasmic tRNAs | [MOD'] | Increased level of ergosterol; resistance to antifungal agents | Crow and Li, 2011; Chernova et al., 2014; Manjrekar and Shah, 2020 |
| Nup100        | FG (phenylalanine and glycine repeats) nucleoporin component of central core of the nuclear pore complex | [NUP100'] | Regulation of nuclear pore complex proteins | Halfmann et al., 2012; Chernova et al., 2014; Manjrekar and Shah, 2020 |
| Pin3          | Negative regulator of actin nucleation-promoting factor activity | [LSB'] | Induction of [PS'] phenotype | Chernova et al., 2017 |
| Vts1          | Flap-structured DNA-binding and RNA-binding protein that stimulates deadenylation-dependent mRNA degradation mediated by the Ccr4-Not deadenlyase complex | [SMAUG'] | Repression of meiosis or the formation of spores | Parfenova and Barral, 2020 |
| Unknown''     | | [NSF']*** | Translational read-through; inhibition of vegetative growth | Crow and Li, 2011 |

"Standard protein names and their molecular functions were retrieved from the Saccharomyces Genome Database (yeastgenome.org). It was hypothesized that the protein determinant of the [NSF'] prion is an interactor of Vts1 protein as the overexpression of the VTS1 gene results in a similar phenotype as [NSF'] prion phenotype. It should be noted that the VTS1 overexpression does not induce the de novo appearance of [NSF'] prion which eliminates Vts1p from the candidates for the protein determinant of [NSF']. It has been shown that the [NSF'] phenotype is determined by two other prions – [SWT'] and [PIN']. Both inhibit SUP45 expression which results in non-sense suppression (Nizhnikov et al., 2016). The Sup45p is the polypeptide release factor (eRF1) which interacts with Sup35p.

The year 2020 may be another breakthrough in studies on yeast prions. The [SMAUG+] prion, or aggregated Vts1p, appears to be a non-amyloid, self-assembling, gel-like particle that hyperactivates its component (Chakravartty et al., 2020). The Vts1 protein binds specific hairpin structures...
within RNA, thus triggering their degradation, specifically transcripts encoding meiotic proteins. The traditional way of understanding prions was to expect that proteins trapped in amyloids have no or limited biological activity. Recent reports regarding [SMAUG] prion revolutionizes our view on the nature of prions.

These examples just strengthen the doubt – is it possible that only yeasts have such a wide variety of prion proteins? Computational analyses bring some insights into this issue. PrionHome is a database of prionogenic sequences (Harbi et al., 2012; online database seems to be discontinued as of April 2020). It predicts prionogenic sequence either by glutamine/asparagine-richness or by using the Hidden Markov Model algorithm successfully implemented by Alberti et al. (2009). In the initial version of PrionHome, there were 20 different mammalian prionogenic sequences registered with more than 400 orthologs of these proteins (Harbi et al., 2012). The other group maintains the PrionScan database which employs the probabilistic model also based on the algorithm by Alberti et al. (2009), which at the time of its publication counted about 650 prionogenic sequences in mammalian proteomes. The PrionScan algorithm predicted also 5460 bacterial, 15,549 invertebrate, 934 plant, and 226 viral prionogenic sequences (Espinosa Angarica et al., 2014). Many observations confirm these predictions – the Rho protein governing the transcription process in Cladostereum botulinum (Yuan and Hochschild, 2017), the CPEB protein in sea slug Aplysia californica (Heinrich and Lindquist, 2011) or CPEB Orb2 in Drosophila melanogaster (Majumdar et al., 2012) engaged in the persistence of memory, and Luminidependens protein controlling the flowering in Arabidopsis thaliana (Chakrabortee et al., 2016) have been demonstrated to display prion properties. To complete this landscape, the discovery of viral protein with prion properties has been looked forward to for a long time. Finally, last year such protein has been found—LEF-10 transcription factor from baculovirus not only has prion properties but also is capable to replace PrD of yeast Sup35 protein (Nan et al., 2019).

These discoveries show that prion and prion-like proteins are not only expected to exist in the wide range of organisms in nature, but they really do exist and probably are waiting to be uncovered. A recent report shows that at least some of the potential prion and prion-like proteins in mammals have been difficult to identify due to the alternative splicing events (Casacrina and Ross, 2020). The next few years will likely deliver a completely different view of prion proteins. Now it became clear that mammalian prion protein is not the only prion protein in multicellular eukaryotes.

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