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Application of yeast to studying amyloid and prion diseases

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Abstract

Amyloids are fibrous cross-β protein aggregates that are capable of proliferation via nucleated polymerization. Amyloid conformation likely represents an ancient protein fold and is linked to various biological or pathological manifestations. Self-perpetuating amyloid-based protein conformers provide a molecular basis for transmissible (infectious or heritable) protein isoforms, termed prions. Amyloids and prions, as well as other types of misfolded aggregated proteins are associated with a variety of devastating mammalian and human diseases, such as Alzheimer's, Parkinson's and Huntington's diseases, transmissible spongiform encephalopathies (TSEs), amyotrophic lateral sclerosis (ALS) and transthyretinopathies. In yeast and fungi, amyloid-based prions control phenotypically detectable heritable traits. Simplicity of cultivation requirements and availability of powerful genetic approaches makes yeast *Saccharomyces cerevisiae* an excellent model system for studying molecular and cellular mechanisms governing amyloid formation and propagation. Genetic techniques allowing for the expression of mammalian or human amyloidogenic and prionogenic proteins in yeast enable researchers to capitalize on yeast advantages for characterization of the properties of disease-related proteins. Chimeric constructs employing mammalian and human aggregation-prone proteins or domains, fused to fluorophores or to endogenous yeast proteins allow for cytological or phenotypic detection of disease-related protein aggregation in yeast cells. Yeast systems are amenable to high-throughput screening for antagonists of amyloid formation, propagation and/or toxicity. This review summarizes up to date achievements of yeast assays in application to studying mammalian and human disease-related aggregating proteins, and discusses both limitations and further perspectives of yeast-based strategies.

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1. Introduction

Protein misfolding in humans and animals have been linked to more than 40 diseases (see Table 1 for examples), including fatal and incurable neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) diseases, as well as diseases affecting other tissues, such as systemic amyloidosis (Knowles, Vendruscolo, & Dobson, 2014). These diseases are typically associated with at least one protein or peptide that misfolds to acquire a so-called amyloid state, in which identical protein molecules are assembled into non-covalent cross-β fibrous polymers, that are typically accumulated in tissues or organs, where the disease-specific damage occurs. Some diseases that were not previously considered as amyloid diseases are now shown to be associated with an amyloid deposition, although it is not always clear if amyloids represent a cause or a consequence of the disease in such cases. Examples of such diseases include type II diabetes, pre-eclampsia and even some forms of cancer (Antony et al., 2012; Buhimschi et al., 2014; Hull, Westermark, Westermark, & Kahn, 2004; Silva, Cino, Soares, Ferreira, & AP de Oliveira, 2018). There are also diseases, such as some forms of Amyotrophic lateral sclerosis (ALS), that are associated with protein misfolding and aggregation but do not necessarily exhibit the formation of "classic" amyloid fibrils (Ayers & Cashman, 2018).

Among protein misfolding diseases, transmissible spongiform encephalopathies (TSEs) or prion diseases were thought to be unique in their ability to be infectious, or in other words, transmissible between organisms (Aguzzi & Lakkaraju, 2015; Colby & Prusiner, 2011; Prusiner, 1998, 2013). TSEs are relatively rare in humans; however, the epidemics of "mad cow," or bovine spongiform encephalopathy (BSE) disease, that is transmissible to humans, demonstrated their importance for public health. The infectious TSE agent is composed of a protein (termed prion protein, or PrP), that is present in a misfolded form, and can initiate and spread the misfolding of an identical substrate protein in the infected organism. The nucleated polymerization of an amyloid explains the infectious capabilities of prions, as the molecules, immobilized into an amyloid fibril, acquire the same conformation as those already included in the fibril, due to the formation of β-strands in the exact same positions (see Fig. 1). This templated mechanism of amyloid polymerization makes any amyloid conformer potentially capable of spreading. Indeed, many amyloids can spread between cells or brain regions within an organism, and intercellular or even inter-organismal transmission of amyloids associated with AD, PD and other synucleinopathies, or HD has been demonstrated in experimental models, thus suggesting a broader use of the term "prion" (Erana, 2019; Jucker & Walker, 2018; Kane et al., 2000; Prusiner, 2012; Prusiner et al., 2015; Ren et al., 2009; Tarutani & Hasegawa, 2019; Walker, 2018; Watts et al., 2013).

The templated mechanism of amyloid reproduction serves as a molecular basis for the inheritance of traits, encoded in the protein structure rather than directly in DNA sequence (Chernoff, 2001; Wickner et al., 2014). Indeed, some endogenous amyloids of yeast and other fungi manifest themselves as heritable non-Mendelian factors transmitted via the cytoplasm (Chernova, Wilkinson, & Chernoff, 2017; Liebman & Chernoff, 2012; Wickner, Edskes, Gorkovskiy, Bezsonov, & Stroobant, 2016). The high resolution power of genetic tools that are available in yeast significantly aids in amyloid characterization, while the ability of yeast prions to cause detectable phenotypic traits (typically associated with a

decrease in cellular function of a protein in an amyloid form) simplifies monitoring of amyloids. Some yeast prions control easily detectable phenotypic traits, typically resulting from a partial loss of the cellular function of a protein because of its incorporation into amyloid polymers.

Some yeast prions are shown to be pathogenic to yeast cells (McGlinchey, Kryndushkin, & Wickner, 2011; Wickner, 2019; Wickner et al., 2011), although the evidence in favor of the adaptive functions of some prions (such as [*Het-s*] prion of the mycelial fungus *Podospora anserina*) has also been provided (Saupe, 2011; Saupe, Jarosz, & True, 2016). In other organisms, certain amyloids have been implicated in biologically positive functions as reviewed in Fowler and Kelly (2012) and Otzen and Riek (2019). Examples include: attachment to substrate or cell-to-cell interactions in bacteria and fungi (Barnhart & Chapman, 2006; Blanco, Evans, Smith, Badtke, & Chapman, 2012; Lipke, Klotz, Dufrene, Jackson, & Garcia-Sherman, 2018), scaffolding of the synthesis of covalent polymers, such as melanin (Fowler et al., 2006), and storage of peptide hormones in animals (Maji et al., 2009). Amyloid-like oligomerization of the CPEB protein has been linked to long-term potentiation and memory in shellfish *Aplysia* (Si, Choi, White-Grindley, Majumdar, & Kandel, 2010; Si, Lindquist, & Kandel, 2003), fruit fly *Drosophila* (Majumdar et al., 2012), and mouse *Mus* (Fioriti et al., 2015). Many proteins or peptides are capable of forming amyloids *in vitro* depending on conditions. It has been proposed that amyloid represents an ancient protein fold that has been suppressed in evolution for a majority of proteins as the amyloid formation interfered with their functions. Thus, amyloid formation *in vivo* is either pathogenic or retained in cases when it plays biologically positive roles.

While a number of approaches have been developed for the prediction of amyloidogenic properties (Antonets & Nizhnikov, 2013, 2017; Conchillo-Sole et al., 2007; Fernandez-Escamilla, Rousseau, Schymkowitz, & Serrano, 2004; Maurer-Stroh et al., 2010; O'Donnell et al., 2011), most of these approaches are capable of accurately predicting amyloid formation by short peptides *in vitro,* but not by full-length proteins in the *in vivo* conditions. The ArchCandy algorithm, based on the ability of an amino acid (aa) sequence to form folded parallel in-register intermolecular β-sheets (termed β-archs), that are characteristic of many amyloids, claims relatively accurate predictions for known proteins (Ahmed, Znassi, Chateau, & Kajava, 2015). However, ArchCandy is yet to be tested in the large-scale searches. An attempt to perform a large-scale bioinformatic search based on several features found in previously confirmed amyloidogenic sequences has uncovered 260 proteins with an amyloidogenic potential in human proteome (Prabakaran, Goel, Kumar, & Gromiha, 2017). While these data are still awaiting experimental validation, they point to a possibility of that amyloid formation is widespread *in vivo.* Either some of these amyloids are functional, or the adverse effects of amyloids are offset by the presence of structural optimization strategies in the proteome. Readers may find a more detailed overview of existing amyloid prediction algorithms in some recent papers (Ahmed & Kajava, 2013; Wilson et al., 2018).

Many amyloid diseases are characterized by late onset (that is, age-dependence) or long incubation periods (Aguzzi & O'Connor, 2010; Colby & Prusiner, 2011; Irvine, El-Agnaf, Shankar, & Walsh, 2008). This makes it extremely difficult to investigate mechanisms of amyloid formation and propagation *in vivo* by using animal models. Yeast provides an

excellent opportunity for amyloid studies due to straightforward cultivation techniques, easiness of producing large cell numbers, and availability of simple and powerful phenotypic assays. As amyloid properties are determined, to a significant extent, by the protein itself, "humanized" yeast cells expressing mammalian amyloidogenic proteins can be employed for studying the fundamental rules of amyloid behavior *in vivo* and searching for anti-prion treatments. This review describes the application of yeast models to the investigation of properties of mammalian amyloidogenic proteins.

2. Overview of yeast prions

Yeast prions were covered in detail in some recent reviews (Chernova, Wilkinson, & Chernoff, 2014; Chernova, Wilkinson, et al., 2017; Cox & Tuite, 2018; Liebman & Chernoff, 2012; Wickner, 2016), thus we summarize only the aspects that are important for understanding their applications to studying mammalian amyloids below. Prions are best studied in the budding yeast *Saccharomyces cerevisiae,* a popular model organism for laboratory research, although some examples of prions from other yeast species and from mycelial fungi have also been reported. Yeast prion proteins contain so-called prion domains (PrDs) that are responsible for intermolecular interactions leading to the formation of an amyloid axis, and are, at least in some cases, distinct from domains responsible for the major cellular functions of the same proteins. Usually PrDs are present as intrinsically disordered, or low complexity regions (IDRs, or LCRs) in the native (non-amyloid) protein structures, and most (although not all) known yeast prion proteins contain PrDs that are enriched by Q and/or N residues.

At present, about 10 yeast proteins are proven to form amyloid-based prions in yeast (see Table 2, for examples). In addition, a variety of QN-rich domains capable of prion formation when fused to reporter constructs were found (Alberti et al., 2009). It was also shown that about 100 yeast proteins possess QN-rich regions similar to known PrDs, and from 1% to 4% of proteins with such regions were found in the proteomes of higher eukaryotes including humans proteomes (Michelitsch & Weissman, 2000). Yeast cells also contain non-QN-rich amyloids, both of prion nature, such as Mod5 (Suzuki et al., 2012), and of non-prion nature, potentially playing a functional role, for example, cell wall proteins Bgl2 (Bezsonov et al., 2013; Kalebina et al., 2008; Selivanova et al., 2016) and Toh1 (Sergeeva et al., 2019). A proteome-wide screening approach for identification of detergent-resistant assemblies (potential amyloids) has been developed for yeast cells and could be applied to mammalian systems (Nizhnikov et al., 2014). Improved bioinformatic searches employing other sequence patterns rather than only QN-richness further increased the numbers of candidate proteins with "prion domain like domains" (PrLDs), both in yeast and in other organisms (Alberti et al., 2009; Iglesias, Conchillo-Sole, Batlle, & Ventura, 2019; March, King, & Shorter, 2016; Pallares et al., 2018; Prabakaran et al., 2017; Zambrano et al., 2015). Moreover, PrLDs (which can be considered as a subgroup of LCRs, low complexity regions that are intrinsically disordered in non-amyloid state) are found in a large number of proteins that are not phylogenetically related to each other and have diverse biological functions. This suggests that PrLDs may have some regulatory roles, although it is not yet known if these roles are related to their amyloid-forming abilities.

The best characterized yeast prion-forming proteins are the translation termination (release) factor Sup35, denoted as [*PSI*$^+$] in its prion form (Cox, 1965; Wickner, 1994), and a posttranscriptional regulator in the nitrogen metabolism, Ure2, denoted as [*URE3*] in its prion form (Lacroute, 1971; Wickner, 1994). The Sup35/[*PSI*$^+$] system, also reviewed in (Cox & Tuite, 2018; Liebman & Chernoff, 2012) is explained in more detail below, as it is used in a variety of assays described in this review. A yeast counterpart of the eukaryotic release factor eRF3, Sup35 consists of three regions (see Fig. 2A): (1) prion domain, or PrD at N-terminus (Sup35N), which contains a QN-rich stretch (NQ), and a region of oligopeptide repeats (NR); (2) middle linker domain (Sup35M), which contains clusters enriched in charged residues, and (3) the C-terminal release factor region (Sup35C, or RF) that is essential and sufficient for Sup35's function in translation termination and for cell viability. Sup35N is typically unstructured but can be converted into a cross-β conformation, thus forming the axis of the amyloid fibril. The NQ stretch of Sup35N is primarily responsible for amyloid aggregation and is typically included in the protected cross-β "core" when Sup35 forms an amyloid, while the repeat region (NR) plays an important role in the propagation of prion state (Osherovich, Cox, Tuite, & Weissman, 2004; Toyama, Kelly, Gross, & Weissman, 2007), possibly through interactions with the chaperone machinery as described below. Sup35N and Sup35M regions are also responsible for the inclusion of Sup35 into reversible liquid droplet or hydrogel assemblies, formed in response to the low pH stress and apparently playing a protective role (Franzmann et al., 2018). The charged clusters within Sup35M region act as pH sensors, facilitating the solubilization of liquid droplets during a recovery from the pH stress. Thus, in normal conditions, Sup35M helps to maintain Sup35 in the soluble state. The relationship between the phase separation pathway leading to liquid droplets, and the Sup35 amyloid formation pathway is not clear. Pre-existing prion aggregates have been reported to antagonize the formation of liquid droplets (Franzmann et al., 2018); however, it is not known which role (if any) phase separation may play in *de novo* prion formation. Overall, the existence of protective assemblies modulated by the Sup35NM region agrees with the previously hypothesized role of the PrD-mediated assemblies in protecting Sup35 protein from degradation during stress (Chernoff, 2007). When Sup35N converts into a prion conformation, it is immobilized into insoluble fibrous aggregates, that results in a reduced ability to access terminating ribosomes, thus causing readthrough of nonsense codons (Liebman & Chernoff, 2012). Specifically designed yeast strains with a premature stop codon, for example, in the gene *ADE1* (the UGA mutation *ade1-14*), are used to detect the Sup35 prion, [*PSI*$^+$] phenotypically by growth on selective medium (for example, the medium lacking adenine, – Ade in case of *ade1-14* reporter) or by color on the complete medium, due to accumulation of the polymerized intermediate in the adenine biosynthetic pathway, conferring red color to the yeast cells (see Fig. 2B). Some strains employ the *ade2-1* UAA allele (instead of *ade1-14*) as a reporter, in combination with a weak tRNA-based UAA-suppressor *SUP16*; this suppressor is not able to cause a detectable readthrough of *ade2-1* in the absence of [*PSI*$^+$], but can do this when translation termination becomes impaired due to the presence [*PSI*$^+$] (Cox & Tuite, 2018; Cox, 1965). The Sup35 PrD-containing constructs, for example, the Sup35NM fragment, readily form amyloid fibers *in vitro* when seeded with amyloid aggregates, thus mimicking the conformational conversion of prion proteins *in vivo* (Glover et al., 1997; Serio, Cashikar, Moslehi, Kowal, & Lindquist, 1999). These *in vitro* produced amyloids can transfect yeast

cells, converting the endogenous Sup35 protein into a phenotypically detectable prion state (King & Diaz-Avalos, 2004; Tanaka, Chien, Naber, Cooke, & Weissman, 2004).

The spontaneous *de novo* formation of the [*PSI*$^+$] prion is very rare, at the rates of 10^{-6} to 10^{-8} depending on strain and prion composition, see refs. (Allen, Chernova, Tennant, Wilkinson, & Chernoff, 2007; Chernoff, Newnam, Kumar, Allen, & Zink, 1999; Lancaster, Bardill, True, & Masel, 2010), and below. However, the frequency of [*PSI*$^+$] can be increased up to 10^{-1} by transient overproduction of the Sup35 protein or its PrD (Chernoff, Derkach, & Inge-Vechtomov, 1993; Derkatch, Chernoff, Kushnirov, Inge-Vechtomov, & Liebman, 1996), see Fig. 3A. Typically, the induction of prion formation by protein overproduction is efficient only in cells that either contain another (typically QN-rich) protein in a prion form (Derkatch et al., 2001, 1997), or co-overproduce another aggregation-prone protein with a QN-rich domain (Derkatch et al., 2001; Osherovich & Weissman, 2001). Specifically, the prion form of the Rnq1 protein, termed [*PIN*$^+$] or [*RNQ*$^+$], promotes *de novo* nucleation of [*PSI*$^+$] by overproduced Sup35 or Sup35N/NM, as well as increases the spontaneous formation of [*PSI*$^+$] (Allen et al., 2007; Cox, Byrne, & Tuite, 2007; Derkatch et al., 2001; Serio, 2018). It was proposed and supported by some data that prion polymers of Rnq1 nucleate the initial assembly of the Sup35 amyloid (Derkatch et al., 2001, 2004), as shown in Fig. 3A. Once formed, Rnq1 and Sup35 amyloids are further maintained as separate entities. *De novo* prion formation by a transiently overproduced prion protein is also promoted by actin cytoskeletal structures that are physically associated with the aggregates of overproduced constructs containing Sup35 PrD (Ganusova et al., 2006). Lsb2 (also called Pin3), a stress-inducible yeast short-lived yeast cytoskeletal protein with a QN-rich domain, also promotes the *de novo* nucleation of [*PSI*$^+$] when Lsb2 is overproduced (Chernova et al., 2011; Derkatch et al., 2001), or when it forms a metastable heat shock inducible prion, termed [*LSB*$^+$] (Chernova, Chernoff, & Wilkinson, 2017; Chernova, Kiktev, et al., 2017). While cross-nucleation interactions between QN-rich proteins are best studied, there are also cases when QN-rich aggregates promote prion formation by a non-QN-rich heterologous protein (Mathur, Taneja, Sun, & Liebman, 2010) or interact with a non-QN-rich yeast amyloid, or when overproduction of a non-QN-rich amyloidogenic protein promotes prion nucleation by Sup35 (Suzuki et al., 2012). In some cases, an aggregated protein apparently promotes prion misfolding and nucleation *in trans* via sequestration of cofactors rather than by direct cross-seeding (Arslan, Hong, Kanneganti, Park, & Liebman, 2015). The *de novo* induction of heritable aggregated state by transient protein overproduction has been used as a tool for the identification of new yeast prion candidates (Alberti et al., 2009; Du et al., 2008; Patel, Gavin-Smyth, et al.2009, , some of which are not QN-rich and are not yet proven to form amyloids (Chakrabortee et al., 2016).

Misfolded proteins are recognized by molecular chaperones that facilitate their folding into native states, as specified by their primary sequence. The Hsp104 chaperone is a homohexameric AAA ATPase, that is required for induced thermotolerance (Glover & Lindquist, 1998). In the context of prions, the levels of Hsp104 expression are crucial for [*PSI*$^+$] propagation: either an overproduction or a deletion of Hsp104 eliminates [*PSI*$^+$] (Chernoff, Lindquist, Ono, Inge-Vechtomov, & Liebman, 1995). Accumulated evidence, reviewed in (Chernova et al., 2014; Chernova, Wilkinson, et al., 2017) demonstrates that Hsp104, together with a cytosolic protein of the Hsp70 family (Ssa) and its Hsp40 cofactor

fragments [*PSI*$^{+}$] fibrils into smaller prion seeds that can efficiently promote the prion conversion of monomeric or newly synthesized Sup35, as shown in Fig. 3B. When Hsp104 levels are depleted, the larger prion fibrils are not fragmented into prion seeds, and thus inefficiently transmitted to daughter cells. On the other hand, an excess of Hsp104 binds prion fibrils independently of Hsp70/40, apparently within the Sup35M region (Helsen & Glover, 2012; Winkler, Tyedmers, Bukau, & Mogk, 2012). This "non-productive" binding does not lead to fragmentation and causes prion loss due to malpartitioning in cell divisions (Ness, Cox, Wongwigkarn, Naeimi, & Tuite, 2017). Some data also point to the ability of excess Hsp104 to solubilize aggregates by "trimming" from the ends, although role of this process in prion "curing" by excess Hsp 104 is not clear (Greene, Zhao, & Eisenberg, 2018; Park et al., 2014). Essentially all known QN-rich yeast prions require Hsp104 for propagation, while they differ in their response to Hsp104 overproduction, with Sup35 being most efficiently cured by excess Hsp104 (Chernova et al., 2014; Chernova, Wilkinson, et al., 2017). The efficiency of polymer fragmentation by chaperones relative to polymer growth explains phenotypic differences between yeast prion variants (Derkatch et al., 1996), analogous to prion or amyloid "strains" in mammalian systems (Rossi, Baiardi, & Parchi, 2019; Tian, Meng, & Zhang, 2019; Vorberg, 2019); notably, the Sup35 "strains" with a longer cross-β protected core are less efficiently fragmented and therefore produce less "seeds," thus exhibiting the "weaker" propagation and phenotype (Tanaka, Collins, Toyama, & Weissman, 2006; Toyama et al., 2007). While Hsp104 is not present in multicellular organisms, other components of the yeast prion fragmentation machinery (Hsp70 and Hsp40) possess orthologs there, as reviewed in (Rikhvanov, Romanova, & Chernoff, 2007). Artificially introduced Hsp104 can antagonize aggregation of some proteins such as polyglutamines in animal cells (Satyal et al., 2000), and "potentiated" variants of yeast Hsp104 with presumably hyperfunctional mutations were shown to antagonize some disease-associated amyloids in both yeast and mammalian systems (Jackrel et al., 2014; Jackrel & Shorter, 2014, 2015). Interestingly, the prion form of yeast Sup35NM can propagate in cultured mammalian cells (Krammer et al., 2009), although it appears that there are some differences between the Sup35 PrD regions that are crucial for the prion propagation in yeast and mammals (Duernberger et al., 2018).

Overall, yeast prions provide an excellent system for understanding the general mechanisms of amyloid formation and propagation, of which many are applicable to mammals. Moreover, availability of powerful phenotypic and cytological assays makes yeast an excellent model for studying properties of specific mammalian and human amyloidogenic proteins, as described in subsequent sections.

3. Yeast models for polyglutamine aggregation

3.1 Overview of polyglutamine diseases

Polyglutamine (polyQ) diseases are neurodegenerative disorders, encompassing at least nine heritable disorders, including Huntington disease (HD) and most spinocerebellar ataxias (SCA) (Shao & Diamond, 2007). In contrast to most other amyloid-type diseases, polyQ diseases are strictly heritable. Each of these diseases results from the expansion of a CAG repeat, coding for a polyQ tract that is present in a respective wild-type protein (for HD, this

is a protein named huntingtin and abbreviated as Htt). However, it has been demonstrated in cell models that huntingtin with a polyQ extension can seed aggregation of wild-type Htt (Ren et al., 2009).

Variations in the size of polyQ tract occur due to replication "slippage" on trinucleotide repeats. In healthy individuals, the length of Htt's polyQ tract is usually below 35 repeats (Fig. 4A). Tracts of intermediate length (between 35 and 40) are termed "premutations," as they may or may not lead to disease, however, individuals with HD frequently appear in the progeny of the carriers of such intermediate expansions. Once the length polyQ tract reaches above 40, it typically results in a disease, and longer tracts (that could be up to over 100 Qs) lead to a more severe disease with earlier onset (Shao & Diamond, 2007). PolyQ expansion promotes formation of fibrous Htt's aggregates, associated with HD. Recombinant proteins with an expanded polyQ stretch were found to form insoluble high molecular weight protein aggregates due to formation of intermolecular polar "zippers" *in vitro* (Perutz, Johnson, Suzuki, & Finch, 1994). PolyQ composition of the aggregation-prone regions in huntingtin aggregates makes them somewhat similar to the endogenous yeast prion proteins, of which many are characterized by the high QN contents (see above). Thus, yeast provides an excellent model for studying the mechanisms leading to polyQ aggregation.

3.2 Factors modulating polyQ aggregation and toxicity in yeast

Yeast models for polyQ-mediated aggregation typically employ exon 1 of huntingtin protein (Chernova, Chernoff, & Wilkinson, 2019; Duennwald, 2013). Exon 1 is coding for the N-terminal region in the wild-type huntingtin protein and includes the polyQ stretch, and the P-rich region following it (Fig. 4A). The length of the protein region encoded by exon 1 with a 23Q stretch (minimal length in a non-altered protein) is 68 aa residues. However, the length of polyQ tract in healthy individuals varies from 23 to 35 aa. Typically the yeast constructs are based on the whole or portion of exon 1 (see below), fused to a C-terminal fluorescent protein tag—most frequently, green fluorescent protein, or GFP (Fig. 4B). Constructs with varying lengths of polyQ stretch, from 25 (corresponding to healthy individuals) to 103 (corresponding to severe early-onset HD) have been produced and tested in yeast. In some Htt-based yeast plasmids, the sequence of the polyQ coding stretch is artificially engineered into the interspersed CAG and CAA triplets, rather than homogenous CAG as in humans (Meriin et al., 2002). This prevents replication slippage, leading to the instability of the repeat length during propagation in yeast. Some constructs (designated here and further as polyQ, Fig. 4B) lack the P-rich region, while other constructs (designated here and further as polyQP, Fig. 4B) include the whole exon 1 with P-rich region. This should be noted that some constructs designated in literature as 103Q (or 103QP) in fact contain somewhat lesser number of Qs (by several residues) within the polyQ tract, possibly because initial designation has also taken into account Q residues located with the P-rich region. However, to avoid confusion, we designate all these constructs here and further as 103Q or 103QP.

The system using exon 1 based constructs is adequate for studying Htt aggregation, as studies in mammalian cells have shown that Htt aggregation is frequently associated with short N-proximal proteolytic Htt fragments (encompassing exon 1) in native conditions (Ratovitski et al., 2009). While aggregates of short fragments may immobilize the full-

length protein, possible impairment of the Htt protein function is apparently irrelevant to the disease mechanism; therefore the presence of the full-length protein is not necessary for studying the mechanisms of polyQ aggregation and toxicity. As in humans, 25Q (or 25QP) Htt exon 1, fused to GFP (designated here and further as Htt-GFP) is soluble in yeast, while expansion of polyQ tract promotes aggregation (Krobitsch & Lindquist, 2000; Meriin et al., 2002; Wang et al., 2009), depending on a strain (see below). It has been shown by cryo-electron tomography that expanded polyQ constructs form both unstructured inclusions and structured fibrillar aggregates in yeast cells (Gruber et al., 2018; Peskett et al., 2018). Notably, yeast studies revealed an important role of P-rich region in determining the mode of Htt aggregation and cytotoxicity, as described below (Gong et al., 2012; Peskett et al., 2018; Wang et al., 2009).

Importantly, aggregation and toxicity of the Htt-GFP constructs with expanded polyQ stretch in yeast cells are promoted by the presence of the endogenous yeast QN-rich prions, such as [*PIN*$^+$], a prion form of the Rnq1 protein (Meriin et al., 2002). The 103Q-GFP construct rarely aggregates in the [*pin*$^-$] cells (lacking the Rnq1 prion), while it forms numerous cytologically detectable dots, leading to cytotoxicity in the [*PIN*$^+$] cells (Fig. 5A). At least some other yeast prions, for example, [*PSI*$^+$], a prion form of the Sup35 protein, can substitute for the Rnq1 prion in regard to promotion of polyQ aggregation. Notably, overexpression of Htt-based constructs with the expanded polyQ region promoted *de novo* formation of [*PSI*$^+$] in the cells lacking the Rng1 prion (Derkatch et al., 2004); thus, expanded polyQ could, to a certain extent, substitute for the [*PIN*$^+$] prion in regard to induction of *de novo* [*PSI*$^+$] formation, even though polyQ aggregation is relatively rare in the absence of [*PIN*$^+$]. As explained above, both Rnq1 and Sup35 proteins contain QN-rich PrDs, making it likely that prion aggregates of these proteins directly nucleate aggregation of the Htt-based polyQ constructs, and *vice versa*.

Interestingly, overproduction of some Q-rich yeast proteins is shown to promote conversion of a fraction of the non-expanded Htt-based construct (25Q-GFP) into insoluble toxic aggregates in yeast (Serpionov, Alexandrov, Antonenko, & Ter-Avanesyan, 2015). On the other hand, overexpression of such yeast proteins with Q-rich PrD-like domains as Gts1, Nab3 and Mcm1 has been shown to antagonize 103Q-GFP toxicity (Ripaud et al., 2014). This occurs due to altering the polyQ interactome rather than by antagonizing polyQ aggregation. Possibly binding of the nonhomogeneous Q-rich sequences to polyQ stretches in protein aggregates prevented them from sequestering other proteins from cytosol.

Toxicity of 103Q-GFP in the [*PIN*$^+$] cells has been linked to the defect in endocytosis (Meriin et al., 2003), possibly because of sequestration of some actin cytoskeleton-associated protein (frequently containing QN-rich domains). Indeed, proteomic characterization of the polyQ-expanded Htt aggregates from yeast cells shows that they accumulate some cytoskeleton-associated and other endogenous QN-rich proteins (Wang, Meriin, Costello, & Sherman, 2007). Some studies also connect Htt aggregation in mammalian cells to the endocytosis defect (Harjes & Wanker, 2003; Meriin et al., 2007; Waelter et al., 2001), thus signifying the relevance of yeast data.

This has been initially reported (Krobitsch & Lindquist, 2000) that aggregation of polyQ-expanded Htt in yeast depends on the chaperone protein Hsp104, shown earlier to modulate propagation of yeast prions (see above). However, later studies demonstrated that at least for the 103Q-GFP construct, this effect of Hsp104 is largely indirect: deletion of the *HSP104* gene impairs propagation of the prion state of the Rnq1 protein, thus converting the [*PIN*$^+$] cells into [*pin*$^-$] cells, in which aggregation of 103Q-GFP is inefficient and toxicity is not detected (Meriin et al., 2002). However, some mutations in Hsp104 modulate polyQ toxicity without eliminating yeast prions (Gokhale, Newnam, Sherman, & Chernoff, 2005). This suggests that some interaction of Hsp104 with polyQ aggregates possibly occurs in yeast. Yeast Hsp70 chaperone, as well as some yeast or mammalian chaperones of the Hsp40 family also influence aggregation and toxicity of the Htt-based polyQ constructs in the yeast model (Muchowski et al., 2000), in an agreement with data obtained in mammalian models (Hageman et al., 2010; Kakkar et al., 2016). Specifically, overexpression of the chaperone protein Sis1 of the DnaJB group decreases size and counteracts cytotoxicity of the 103Q-GFP aggregates in yeast (Gokhale et al., 2005), and the human chaperone DnaJB6 (Kumar, Kline, & Masison, 2018) of the same family also counteracts polyQ cytotoxicity when expressed in yeast cells. Overexpression of Sis1 is also shown to counteract toxicity of other protein aggregates in yeast, as described below and in Park et al. (2018, 2017). In contrast, some other mammalian DnaJB chaperones don't have such an effect, while overproduction of the yeast Hsp40 chaperone of the DnaJA family, Ydj1 exhibits an opposite effect, leading to an increase of both aggregate size and cytotoxicity of 103Q-GFP (Gokhale et al., 2005). Deletions of the genes coding for two other Hsp40 chaperones, Apj1 and Hlj1, were also shown to increase toxicity of the Htt-derived 53Q construct, expressed in yeast cells, and in case of Hlj1, this effect of the respective deletion was partly rescued by expression of its human ortholog, DnaJA2 (Willingham, Outeiro, DeVit, Lindquist, & Muchowski, 2003). It still remains to be understood if effects of Hsp40 chaperones on polyQ aggregation are direct, or mediated by an endogenous prion present in yeast cells. In case of Sis1, it has been shown that expanded polyQ aggregates sequester Sis1 chaperone in the yeast cells, that inhibits proteasome-mediated degradation of other yeast proteins; this sequestration may at least partly explain the antitoxic effect of excess Sis1 (Park et al., 2013). Interestingly, the multisubunit chaperonine complex (TriC or Cct) is involved in the modulation of interactions between yeast chaperones and polyglutamine aggregates, and increased abundance of TriC is antitoxic, possibly by directing polyQ polymerization toward formation of non-toxic oligomers (Behrends et al., 2006).

The yeast deletion library was used to search for gene deletions that suppress toxicity of 103Q-GFP (Giorgini, Guidetti, Nguyen, Bennett, & Muchowski, 2005). However, it has been shown later that some deletion derivatives uncovered by this screen have lost [*PIN*$^+$] prion that is required for 103Q-GFP toxicity (Manogaran, Fajardo, Reid, Rothstein, & Liebman, 2010). The loss of [*PIN*$^+$] apparently was a by-product of the deletion construction procedure, including the anti-prion treatments such as osmotic stress (used in the transformation techniques) *etc.*, thus it was not related to the deletion *per se* and produced a false positive in the screen. Nevertheless, at least some deletion strains, identified in the initial screen, retained [*PIN*$^+$]. One of them contained a deletion of the gene that encodes Bna4 (kynurenine 3-monooxygenase, KMO), an enzyme in the kynurenine pathway of

tryptophan degradation, also implicated in the pathology of HD in humans (Jacobs, Castellano-Gonzalez, Guillemin, & Lovejoy, 2017). Moreover, a small molecule inhibitor of KMO, Ro61-8048, rescued 103Q-GFP mediated toxicity in yeast (Giorgini et al., 2005). KMO and the kynurenine pathway are being now proposed as respectively a potential drug and a drug target for HD (Campesan et al., 2011; Jacobs et al., 2017; Wild & Tabrizi, 2014; Zwilling et al., 2011), further emphasizing the disease relevance of the yeast model. Other small molecule compounds counteracting polyQ aggregation and/or toxicity that have been isolated by using yeast models are reviewed in Chernova et al. (2019).

Another interesting observation is that a construct with a moderate expansion of the polyQ stretch, 56Q, causes a cytostatic effect (that is, prevents cell division) of the haploid yeast cells, but is not toxic to diploids and cells of higher ploidy levels (Kaiser et al., 2013). The 56Q-arrested haploid cells exhibit mislocalization of the septins Cdc10 and Shs1, suggesting their involvement in the polyQ toxicity, at least in dividing cells. Relevance of this observation to HD is so far unclear, as neurons are not dividing.

3.3 Role of P-rich stretch and aggresome formation

Importantly, the presence of the P-rich sequence, immediately following the polyQ tract within exon 1 of Htt, ameliorated cytotoxicity and changed the mode of aggregation of the expanded polyQ constructs in yeast cells, containing the [*PIN*$^+$] prion (Wang et al., 2009). In contrast to 103Q-GFP, producing numerous dot-like aggregates, the 103QP-GFP protein typically aggregated into a single large clump as shown in Fig. 5B. This assembly was different from the so-called JUNQ, a perinuclear deposit of soluble misfolded proteins (Kaganovich, Kopito, & Frydman, 2008), as the 103QP-GFP clump contained detergent-resistant insoluble polymers (Gong et al., 2012). Thus, it rather resembled another deposit of the insoluble misfolded proteins, previously observed in yeast in the conditions of proteolysis impairment, and termed IPOD (Kaganovich et al., 2008). However, IPOD typically shows peripheral localization, while 103QP-GFP deposit was perinuclear at least in some cells and was colocalized with the spindle body, or SPB (yeast microtubule organizing center, analogous to mammalian centrosome), Spc72 (Wang et al., 2009). Colocalization of the SPB marker with the peripheral IPOD-like structure formed by Rnq1 protein upon its overproduction has also been reported by another group (Treusch & Lindquist, 2012), however, such an IPOD-like formation was toxic, likely due to sequestration and misplacement of the SPB components. Contrary to this, the formation of the 103QP-GFP deposit was cytoprotective in the yeast [*PIN*$^+$] cells (Wang et al., 2009), showing that the SPB assembly and organization remained unaltered. Apparently, SPB acts as an “assembly center” for 103QP-GFP, rather than being sequestered by 103QP-GFP aggregates. In both its localization and protective functions, the 103QP-GFP deposit resembled mammalian perinuclear aggregate of misfolded proteins (including Htt), termed an aggresome (Johnston, Ward, & Kopito, 1998; Kopito, 2000). Here and further, we refer to this aggregate as yeast aggresome, although it is possible that the aggresome and IPOD deposits are formed by similar mechanisms in yeast cells, and just differ by location in specific cases. Notably, the defect in endocytosis was not detected in the [*PIN*$^+$] cells expressing 103QP-GFP and containing an aggresome (Gong et al., 2012; Wang et al., 2009), in contrast to those expressing 103Q-GFP and bearing multiple aggregates. Apparently, assembly of 103QP-

GFP into an aggresome impaired the ability of polyQ stretches to interact with cytoskeleton-associated proteins and sequester them, thus leading to a cytoprotective effect.

An exact mechanism by which a P-rich region promotes the aggresome assembly remains unclear. Possibly, the presence of the structure-breaking P-rich sequence immediately next to the cros-μ-forming polyQ core serves as a signal for the recognition by proteins promoting the assembly of the aggresome deposit. Detailed analysis employing electron microscopy techniques demonstrated that the P-rich region in a combination with expanded polyQ tract facilitates formation of the liquid-liquid phase separation assemblies, which can then be converted into irreversible fibrillar aggregates (Peskett et al., 2018).

Notably, the P-rich region may promote aggresome assembly *in trans,* when it is fused to 25Q stretch and expressed in the presence of 103Q-GFP aggregates. This agrees with the observations that Htt aggregates with an expanded polyQ stretch and Htt derivatives with a non-expanded stretch are co-assembled into the same aggregates in mammalian cells. In addition to the P-rich stretch, the N-terminal 17 aa residues of exon 1 are also important for the aggresome formation (Wang et al., 2009). Interestingly, the yeast model demonstrated that the expression of the wild-type exon 1 lacking the P-rich region counteracts aggregation of polyQ-expanded exon 1, as well as aggregation of some mammalian proteins lacking polyQ stretches (Sethi et al., 2018). It appears that the Htt protein contains sequences capable of ameliorating the aggregation and/or toxicity caused by the polyQ expansion.

Yeast model is well suited for the identification and characterization of the proteins involved in the control of aggresome assembly and integrity. The Cdc48 protein (a member of the AAA + superfamily and distant paralog of Hsp104), and a member of the pleiotropic 14-3-3 chaperone family, Bmh1, as well as microtubular cytoskeleton (as shown by the effect of an anti-microtubule drug benomyl) are implicated in the aggresome assembly (Wang et al., 2009). It is also shown that downregulation of one of the essential genes, *RVB1* or *RVB2* leads to the formation of numerous small aggregates instead of a single aggresome by 103QP-GFP, and makes it toxic to the [*PIN*$^+$] yeast cells (Zaarur et al., 2015). *RVB1* and *RVB2* code for the proteins of AAA + superfamily, orthologous to mammalian RuvbL1 and RuvbL2, respectively (Matias et al., 2015). Downregulation of the RuvbL1 or RuvbL2 production impairs the polyQ-derived aggresome formation in mammalian cells as well (Zaarur et al., 2015). Importantly, RuvbL1 and RuvbL2 exhibit protein disaggregation activity, both *in vivo* (Narayanan et al., 2019) and in regard to amyloid β (Aβ, see Table 1 and below) fibrils, *in vitro* (Zaarur et al., 2015). It is possible that RuvbL/Rvb proteins promote aggresome formation via disassembly of polyQ aggregates into oligomers, that are recognized by the aggresome-forming machinery as substrates for the aggresome assembly, while larger aggregates cannot be transported to the aggresome site and become toxic.

Another factor that makes the 103QP-GFP expression toxic to yeast cells is the presence of the prion [*PSI*$^+$] (Gong et al., 2012), as shown in Fig. 5B. An aggresome is still formed in the [*PSI*$^+$] cells; however, it is no longer cytoprotective. As mentioned above, [*PSI*$^+$] is a prion form of the translation termination factor Sup35 (eRF3). It is shown that an aggregated form of Sup35, sequestered by the 103QP-GFP aggregates, in turn mediates sequestration of another translation termination factor, Sup45 (eRF1) (Gong et al., 2012; Zhao et al., 2012),

which is a normal interacting partner of Sup35. Increase in the Sup45 levels counteracts cytotoxicity of 103QP-GFP. Depletion of Sup45 from the terminating ribosomes causes a cytotoxicity. 103Q-GFP aggregates lacking the P-rich region can also sequester some fraction of the Sup35 and Sup45 proteins in the [*PSI*$^+$] strain, that apparently contributes to 103Q-GFP toxicity, in addition to the endocytosis defect described above (Kochneva-Pervukhova, Alexandrov, & Ter-Avanesyan, 2012). Sequestration of release factors *per se* is not likely to be directly applicable to humans, as human ortholog of Sup35 does not contain the QN-rich region, capable of mediating interac-tions with polyQ aggregates (Inge-Vechtomov, Zhouravleva, & Philippe, 2003). However, these data could be still relevant to HD in a more general sense, as it has been demonstrated that polyQ aggregates or oligomers may sequester some other components of translational machinery in fruit flies or mammalian cells (Joag et al., 2019; Kim et al., 2016).

Overall, the yeast model for HD shows that the composition of endogenous aggregated proteins serves as a major modulator of Htt aggregation and toxicity at least in yeast (and possibly in humans). The presence of endogenous amyloids/prions determines both the mode of polyQ aggregation (cytotoxic versus cytoprotective) and the composition of proteins, sequestered by polyQ aggregates. Therefore, both the prion composition of the cell, and the type of the Htt construct have to be taken into account when results of the experiments, using Htt-based polyQ constructs in yeast are interpreted. In application to humans, it is possible that the variability in the composition of endogenous protein aggregates between different cell types could explain why the Htt aggregates are toxic primarily to neurons, while the differences in endogenous protein aggregation between neurons from different individuals could be responsible for the largely “non-genetic” variation in HD onset, reported previously (Wexler et al., 2004).

4. Yeast models for aggregation of α-synuclein, associated with Parkinson’s disease (PD)

PD is associated with intracellular aggregation of the 140-aa intracellular protein termed α-synuclein (αSyn) (Uversky, 2017). In contrast to HD, most cases of PD are of sporadic (that is, non-genetic) nature, also some heritable forms of PD have also been identified (some of them are caused by mutations in αSyn). While yeast cells do not have an ortholog of αSyn, human αSyn can be expressed in yeast, either alone or in fusion to a fluorophore such as GFP. High levels of αSyn expression result in its aggregation, accompanied by growth inhibition and cytotoxicity in a dosage-dependent fashion (Outeiro & Lindquist, 2003). However, in contrast to polyQ aggregates, expression of αSyn (as well as of some other non-QN-rich aggregating proteins, such as synphilin and transthyretin) does not promote aggregation and prion formation by Sup35 (Derkatch et al., 2004). This indicates that αSyn and at least some other non-QN-rich aggregates possess features that are distinct from the endogenous yeast prion aggregates. Applications of yeast system to studying PD and related synucleinopathies are summarized in recent reviews (Bras, Popova, Braus, & Outeiro, 2019; Cronin-Furman, Barber-Singh, Bergquist, Yagi, & Trimmer, 2019; Piotrowski & Tardiff, 2019), thus we mention only some selected examples below.

Importantly, yeast studies point to the differences in the mechanisms of polyQ and αSyn toxicity. When the yeast deletion collection was screened for deletions of non-essential genes that increase toxicity of expanded Htt-derived polyQ (53Q) or αSyn constructs, expressed in yeast, 52 deletions increasing sensitivity to 53Q and 86 deletions increasing sensitivity to αSyn have been identified. Among those, only one deletion (that of the gene *STD2*, coding for a transcription factor) increased sensitivity to both (Willingham et al., 2003). Moreover, the sample of genes influencing the sensitivity to αSyn has been enriched in those coding for proteins involved in vesicle trafficking and lipid metabolism. Such genes were not abundant among those influencing sensitivity to 53Q. Such a relationship between the αSyn-mediated toxicity and membrane-related pathways indicates that an association of αSyn with membranous structures (including vesicles) is apparently maintained in yeast and contributes to its toxicity. Roles of the proteasomal and autophagy system in the clearance of misfolded αSyn in yeast (Popova, Kleinknecht, & Braus, 2015), as well as the role of the Hsp70 chaperone Ssa in the reduction of αSyn toxicity via promotion of its degradation by autophagy (Gupta et al., 2018) are also being studied.

Expression of αSyn makes yeast cells more sensitive to hydrogen peroxide, pointing to the connection between α-synucleinopathies and oxidative damage (Liang et al., 2008). Possibly, aggregation of αSyn increases accumulation of reactive oxygen species (ROS), thus making yeast cells incapable of sustaining further increase in the ROS levels. Forty yeast genes capable of suppressing this super-sensitivity phenotype upon overexpression have been identified in a genetic screen. Products of these genes are involved in ubiquitin-dependent protein catabolism, protein biosynthesis, vesicle trafficking and the response to stress. Deletions of each of five genes with the strongest effect (*ARG2, ENT3, HSP82, IDP3* and *JEM1*) increased toxicity of wild-type αSyn and promoted ROS accumulation in the presence of αSyn. Interestingly, most of the strongest suppressors of the toxicity of wild-type αSyn did not rescue yeast cells from the toxicity of αSyn containing a mutation associated with heritable PD, A30P or A53T, even though this has been shown that mutant αSyn also causes increased ROS accumulation (Flower, Chesnokova, Froelich, Dixon, & Witt, 2005). Another study identified a high copy suppressor of the toxicity of wild-type αSyn, Ypt1 that also rescued cells from A53T αSyn, but not from A30P αSyn (Cooper et al., 2006). Wild-type and A53T (but not A30P) αSyn transits through the yeast secretory pathway and is targeted to the plasma membrane (Dixon, Mathias, Zweig, Davis, & Gross, 2005), which may partly explain the differences in the effects of Ypt1, but not in the effects of other suppressors. In contrast, the overproduced Ypp1 protein suppresses toxicity of A30P αSyn, but not of wild-type or A53T αSyn (Flower et al., 2007). Notably, tagging of wild-type αSyn with GFP has shown that overexpression of Ent3 causes relocation of a fraction of αSyn from the peripheral region, underlying plasma membrane to the intracellular puncta (Liang et al., 2008). One possibility is that Ent3, which is a yeast counterpart of the human protein epsin R and is involved in clathrin-mediated retrograde protein transport between Golgi and endosomes, promotes trafficking of wild-type αSyn to endosome for eventual degradation. Likewise, excess Ypp1 is shown to promote trafficking of both wild-type and mutant αSyn from plasma membrane to endocytic vesicles, however, only the A30P αSyn containing vesicles merge to vacuole, where A30P is degraded (Flower et al., 2007). Overall, these data point to the role of endocytic trafficking and stress response

in the amelioration of αSyn toxicity, as well as to the differences in the specific pathogenic mechanisms involved in heritable and sporadic forms of PD.

Yeast system was successfully used for identifying several potential therapeutic candidates, that rescue αSyn aggregation and/or toxicity (Tardiff & Lindquist, 2013). These include some flavonoids (*e.g.,* quercetin and epigallocatechin gallate) (Griffioen et al., 2006), small molecule stimulators of the Rab GTPase, associated with PD (Fleming, Outeiro, Slack, Lindquist, & Bulawa, 2008), 1,2,3,4-tetrahydroquinolinones (Su et al., 2010, cyclic peptides (Kritzer et al., 2009), mannosylglycerate, originated from marine organisms (Faria et al., 2013), red pigment which is a polymerized intermediate in the yeast adenine biosynthesis pathway (Nevzglyadova et al., 2018), ascorbic acid which is a natural antioxidant (Fernandes et al., 2014), and N-aryl benzimidazole (NAB), that promotes endosomal transport via the E3 ubiquitin ligase, Rsp5 (a yeast ortholog of mammalian Nedd4) and apparently antagonizes the vesicular traffic disruption by αSyn (Tardiff et al., 2013). Many of these compounds (including NAB) also turned active in the animal models of PD, as reviewed in (Chernova et al., 2019). These data emphasize the utility of the yeast models for identifying potential PD cures. One general issue with some of αSyn studies using yeast that a distinction between the role of αSyn production and its aggregation is not always clearly made.

5. Yeast models for amyloid proteins associated with Alzheimer's disease (AD) and tauopathies

5.1 Overview of protein aggregation in AD

AD is a fatal and incurable disease, characterized by the progressive loss of neurons, resulting in dementia and eventually in death. It is typically reported as the sixth most frequent cause of death in the United States; however, AD was routinely underdiagnosed in the past, and its evaluation as the third most frequent cause of death in the United States, and possibly in other developed countries with a long life-expectancy is likely to be more realistic (Alzheimer's, 2016; James et al., 2014). AD is one of the major factors affecting the quality of life at an advanced age and is associated with tremendous healthcare costs. The most common form of AD is late onset sporadic AD (with patient age greater than 65 years), while early-onset heritable cases (with the patient age between 30 and 65 years) account for approximately 1–6% of all cases (Alonso Vilatela, Lopez-Lopez, & Yescas-Gomez, 2012). Both extracellular amyloid plaques, formed by amyloid beta (Aβ) and neurofibrillary tangles (NFTs) formed by the intracellular microtubule-associated protein tau (usually called MAPT or tau) in an amyloid form are diagnostic hallmarks of AD (Irvine et al., 2008), and are included in the definition of AD for research purposes, as recommended by the National Institutes of Health and Alzheimer's Association (Walker, Lynn, & Chernoff, 2018).

Aβ (Fig. 6A) is generated via cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. This cleavage produces extremely hydrophobic peptides that include Aβ40, Aβ42, and less abundant Aβ39 and Aβ43 (Irvine et al., 2008), where numbers reflect sizes in aa residues. Aβ42 and less abundant Aβ43 are found to be more prone to aggregation and

more neurotoxic (Irvine et al., 2008; Jarrett, Berger, & Lansbury, 1993; Naslund et al., 1994).

Tau protein (Fig. 6B) is aggregated into bundles of paired helical filaments (PHFs), forming neurofibrillary tangles in AD brains (Irvine et al., 2008; Mietelska-Porowska, Wasik, Goras, Filipek, & Niewiadomska, 2014). Aggregation of tau protein is also detected on other neurodegenerative disorders (termed tauopathies), that include Pick disease and some forms of frontotemporal dementia (FTD), such as FTD with parkinsonism, FTDP-17 (Goedert, 2018; Spires-Jones, Stoothoff, de Calignon, Jones, & Hyman, 2009). Most non-AD tauopathies are heritable and are associated with mutations in a gene, coding for the tau protein (Hutton et al., 1998; Ingram & Spillantini, 2002; Spillantini et al., 1998). Tau is a major microtubule-associated protein present in mature neurons (Gendron & Petrucelli, 2009), and is also implicated in other processes such as formation of stress granules (SGs), protective RNA-protein complexes generated in the cytosol during stress (Maziuk et al., 2018; Vanderweyde et al., 2016). Amyloid-forming core of tau filaments is located within the microtubule-binding repeat region (Fitzpatrick et al., 2017), which is polymorphic due to alternative splicing: isoforms containing from two to four repeats are detected (Andreadis, Brown, & Kosik, 1992; Goedert & Jakes, 1990). In the longest isoform with 4 repeats, the region of repeats is located between aa positions 244 and 372. The four-repeat isoform of tau forms tangles in AD brains, although amyloids, formed by shorter isoforms, are found in some tauopathies (Falcon, Zhang, Murzin, et al., 2018; Falcon, Zhang, Schweighauser, et al., 2018; Goedert, 2018). Phosphorylation of tau modulates its microtubule-binding affinity and in doing so regulates the morphology of neurons and intracellular transport; however, the hyperphosphorylation of tau depresses this biological activity of tau (Gendron & Petrucelli, 2009). In AD and tauopathies, aggregated tau protein is hyperphosphorylated, although it is not clear if hyperphosphorylation plays a causative role in amyloid formation or represents a consequence of the decreased access of protein phosphatases to amyloid fibrils. Mutations, associated with tauopathies are usually located within or near the repeat region, and are shown to reduce ability of tau to interact with microtubules, and/or to lead to overproduction of tau isoforms with four repeats, and/or to stimulate formation of an amyloid by tau repeat fragment *in vitro* (Hutton et al., 1998; Ingram & Spillantini, 2002; Poorkaj et al., 1998; Spillantini et al., 1998). It has been shown that the patient brain extracts can seed *in vitro* aggregation of the construct containing the repeat region of tau protein (Metrick et al., 2019), although some patterns of aggregates produced *in vitro* differ from those used as a seed (Nam & Choi, 2019).

The amyloid cascade hypothesis (Aβ hypothesis) suggesting a causative role of Aβ in AD etiology has been the mainstream explanation for the pathogenesis of AD for over 25 years (Hardy & Selkoe, 2002; Irvine et al., 2008; Walker et al., 2018). According to this model, polymerization of Aβ initiates the pathway to a disease, and induces (directly or indirectly) aggregation of tau, that leads to pathological consequences. Accumulating experimental evidence from *in vitro* models, *in vivo* models, and from biomarkers analysis in patients generally supports the amyloid cascade model. Essentially all mutations associated with the familial (heritable) form of AD occur either within Aβ sequence, or in the flanking regions of APP, or in the genes that control proteolytic processing of Aβ from APP, such as components of the β-secretase complex (Irvine et al., 2008). APP mutations associated with

familial (heritable) AD and falling within the Aβ sequence have been reported to increase the amount of produced Aβ, increase the ratio of Aβ42 to Aβ40, increase the aggregation potential of the mutant Aβ variant, or promote the formation of particularly toxic conformations of aggregates, such as oligomers. The *APOE4* allele, which is the major risk factor for sporadic AD as indicated by genomic studies, influences Aβ aggregation and clearance (Liu et al., 2017; Uddin et al., 2019). A substitution at aa position 2 of the Aβ sequence, that is frequently found in the population of Iceland, leads to a decrease in both Aβ levels and incidence of sporadic AD, as well as to an increase of life span (Jonsson et al., 2012). Promotion of tau aggregation by Aβ aggregates is shown in the cell culture (Ferrari, Hoerndli, Baechi, Nitsch, & Gotz, 2003) and transgenic animal models (Gotz, Chen, van Dorpe, & Nitsch, 2001). While the typical Aβ deposits are extracellular, and tau tangles are intracellular, several studies point to the existence of intracellular Aβ, which might even be formed before extracellular Aβ in the disease development pathway (Wirths, Multhaup, & Bayer, 2004). Likewise, some tau is found outside of the cells, *e.g.,* see (Holmes & Diamond, 2014). Importantly, aggregates of Aβ and tau can self-propagate and spread, both in culture models and throughout the brain (and in experimental models, even between animals) by prion-like mechanisms (Frost, Jacks, & Diamond, 2009; Holmes & Diamond, 2014; Jucker & Walker, 2018; Kane et al., 2000; Kaufman, Thomas, Del Tredici, Braak, & Diamond, 2017). Counterarguments against the amyloid cascade model are primarily based on numerous failures of therapeutic interventions targeting Aβ (Castellani, Plascencia-Villa, & Perry, 2019), however, these failures could be easily predicted from the amyloid model, because none of these interventions specifically addressed of either Aβ conversion into an amyloid or promotion of the tau amyloid formation by Aβ. Moreover, recent immunotherapies using Aβ-aimed antibodies do show some promise in clinical trials (Tolar, Abushakra, & Sabbagh, 2019).

5.2 Yeast models for Aβ

Yeast does not have an APP homolog. While exogenous Aβ shows some toxicity when uptaken by yeast cells (Bharadwaj, Waddington, Varghese, & Macreadie, 2008), relevance of this observation to AD is unclear, because Aβ is generally known to exhibit anti-microbial properties (Soscia et al., 2010). Yeast cells with heterologously expressed Aβ typically don't accumulate it at high levels, possibly due to proteolytic instability of a short peptide in the yeast cytosol, *e.g,* see (Chandramowlishwaran et al., 2018). Thus, most yeast models for studying Aβ are based on chimeric constructs that include Aβ region. The major models are described below.

5.2.1 Fusion of Aβ to a fluorophore—Aβ40 or Aβ42, fused to a fluorophore such as green (GFP), yellow (YFP) or cyan (CFP) fluorescent protein (Fig. 7A), has been expressed and shown to aggregate in yeast. Some constructs allowed microscopic detection of dot or clumps corresponding to Aβ-based aggregates in yeast (Rubel, Ryzhova, Antonets, Chernoff, & Galkin, 2013). This has been shown that aggregated Aβ is present in the form of detergent-resistant polymers as typical of yeast amyloids. The GFP-Aβ construct has also been prepared, in which amyloid formation by Aβ suppressed green fluorescence (Caine et al., 2007; Macreadie et al., 2008). This construct was used to search for compounds that increase fluorescence, in a hope that such compounds would antagonize aggregation and

therefore lead to the development of new drugs against AD. Pentapeptides corresponding to the portions of the hydrophobic core region of Aβ, such as KLVFF or LVFFA, were produced by rationale design and shown to antagonize GFP-Aβ aggregation, decrease GFP-Aβ toxicity and promote GFP-Aβ clearance by autophagy in the yeast cells (Rajasekhar, Suresh, Manjithaya, & Govindaraju, 2015). Similar effects (that is, decreased toxicity and increased clearance) were reported for the anti-histamine drug latrepirdine, that upregulates vacuolar (lysosomal) activity and trafficking to the vacuole (Bharadwaj et al., 2012). This compound also promotes Aβ autophagy in mice (Doody et al., 2008) and shows some benefits in AD trials (Steele et al., 2013). Folinic acid was also uncovered as a compound antagonizing Aβ misfolding in the yeast screen (Macreadie et al., 2008).

5.2.2 Use of a secretory Aβ-based construct—Another yeast mode, also employing Aβ fused to a fluorophore, attempted to recapitulate the Aβ secretion and endocytosis that are observed in human brains (D'Angelo et al., 2013; Matlack et al., 2014). In this model, Aβ42 was fused to either the endoplasmic reticulum targeting signal (ssAβ42-GFP) (Matlack et al., 2014), or to the signal peptide of α-factor, yeast excreted mating pheromone (MFa) (MFα-Aβ42-GFP) (D'Angelo et al., 2013). Accumulation of the Aβ-based constructs in the secretory pathway and/or in the periplasmic space resulted in cytotoxicity. Several metal-binding compounds related to clioquinol (CQ), that is working in the mouse AD model (Cherny et al., 2001) were shown to antagonize toxicity in the yeast screen via promoting Aβ turnover, restoring vesicle trafficking and protecting against oxidative stress (Matlack et al., 2014). The major disadvantage of this assay is that it is targeting Aβ42 accumulation and secretion, so that the toxicity effect might not be relevant to toxicity in human brains. It has been reported that ssAβ42-GFP is present in the detergent-resistant form (Matlack et al., 2014); however, it is not clear if toxicity of ssAβ42-GFP, detected in the yeast assay, is a consequence of its aggregation. While compounds identified in this assay could still be effective in counteracting Aβ accumulation, they do not necessarily target Aβ oligomerization and aggregation, a triggering factor in AD.

5.2.3 Substitution of Sup35 PrD by Aβ—The abovementioned models are based on the detection of aggregation and/or cytotoxicity of Aβ. The model allowing for phenotypic detection of Aβ aggregation by growth or color was produced on the basis of yeast Sup35 prion protein (Park et al., 2011; von der Haar, Josse, Wright, Zenthon, & Tuite, 2007), which is a translation termination factor as described above. In this model, Aβ42 was substituted for the region coding for Sup35N domain, that is Sup35 PrD (Fig. 7B). Resulting construct was termed Aβ-MRF, where M is a middle domain and RF is a release factor, or C domain. Retention of the RF domain allowed its use as a reporter, thus employing the nonsense-suppression assay in the same way as described above for the Sup35 prion. Aβ-MRF instantly oligomerized in yeast, as confirmed by both phenotypic assay and biochemical detection of detergent-resistant polymers using semi-denaturing detergent agarose gel electrophoresis, SDD-AGE. A triple aa substitution with the Aβ region that is predicted from *in vitro* studies to knock out amyloid formation by Aβ antagonized oligomerization and suppression in yeast. Overexpression of the yeast protein Yap1802 also reduced oligomerization and suppression by Aβ42-MRF in yeast (Park, Ratia, Ba, Valencik, & Liebman, 2016). Yap1802 is a yeast ortholog of human phosphatidylinositol binding clathrin

assembly protein (PICALM). Polymorphism in the PICALM-coding gene has been linked to the risk of AD on the basis of the genome-wide association studies in humans, and the deletion of both yeast genes coding for PICALM homologs, *YAP1801* and *YAP1802,* rescued toxicity in the previously described yeast assay for secretory Aβ, while expression of the mammalian PICALM protein in such a double deletion strain partly restored it (D'Angelo et al., 2013), pointing to some similarities between these two yeast Aβ models. The Aβ-MRF construct has been employed to identify compounds reducing Aβ oligomerization from the small molecule libraries and from the list of drugs that are approved by Food and Drug Administration for other purposes (Park et al., 2011, 2016). Notably, the drugs identified by this screen also reduced Aβ42 toxicity to cultured human cells. Therefore, proposed approach is capable of identifying potential anti-AD drug candidates and is amenable to high-throughput screening. The major shortcoming of this assay (as well as other yeast assays for Aβ described above) is that due to instant oligomerization of Aβ-MRF in the yeast cells, it is not possible to target the switch between the monomeric and polymeric forms of Aβ, a step that apparently triggers the development of AD. A yeast model allowing for monitoring of such a switch in various mammalian proteins, including Aβ has been developed recently and is described below (see Section 9). A summary of yeast models for studying Aβ aggregation is provided in Table 3.

5.3 Yeast models for tau

Yeast does not have an ortholog of tau, however, human tau protein has been produced in yeast cells and shown to form detergent-insoluble aggregates, as reviewed in (De Vos et al., 2011). These aggregates were recognized by the conformational antibody MC1 (Vandebroek et al., 2005), capable of binding the pathological tau filaments and their precursors in mammalian cells (Jicha, Bowser, Kazam, & Davies, 1997; Weaver, Espinoza, Kress, & Davies, 2000). Major tau-phosphorylating kinases Gsk-3β (Flaherty, Soria, Tomasiewicz, & Wood, 2000; Ishiguro et al., 1993) and Cdk5 (Flaherty et al., 2000; Kobayashi et al., 1993), and tau-dephosphorylation modulator, Pin1 (Legname et al., 2018; Zhou et al., 2000) possess orthologs in *S. cerevisiae* (Mds1, Pho85 and Ess1, respectively). Indeed, AD-associated phosphorylation epitopes of tau are shown to be phosphorylated in yeast, and hyperphosphorylation at some sites coincides with an increased aggregation (Vandebroek et al., 2005). Phosphorylation was antagonized by a deletion of the *MDS1* gene; however, the deletion the *PHO85* gene increased the proportion of phosphorylated tau. Authors (De Vos et al., 2011) interpret this as an evidence of that at least in yeast, Pho85 (Cdk5) might not directly phosphorylate tau but rather influence tau phosphorylation by Mds1 (Gsk-3β), and point to the other results indicating that in mammalian cells, Cdk5 may interfere with the activity of Gsk-3β (Wen et al., 2008). Indeed, genetic studies show that Mds1 operates downstream of Pho85 in the tau phosphorylation pathway in yeast (Vanhelmont et al., 2010). Hyperphosphorylated tau from the *pho85Δ* cells exhibited increased aggregation propensity, supporting the role of hyperphosphorylation in aggregation (Vandebroek et al., 2005). Tau hyperphosphorylation has also been detected in the strain with impaired activity of Ess1, an ortholog of Pin1 as per data quoted in (De Vos et al., 2011). Counterintuitively, the tauopathy (FTDP-17)-associated mutations P301L or R406W reduced both tau phosphorylation at the S409 site and level of insoluble aggregates, especially in the *pho85Δ* strain (Vanhelmont et al., 2010). This suggests that mechanisms of tau aggregation in

heritable tauopathies could be different from those involved in aggregation of wild-type tau. The substitution of serine by a non-phosphorylated alanine, S409A decreased tau aggregation in yeast, while the phosphorylation-mimicking substitution S409E either increased (in *pho85Δ* cells) or did not change (in wild-type cells) proportion of aggregated tau. By using these mutations, this has also been shown that tau phosphorylation at S409 primes phosphorylation at some other sites. While binding of tau to yeast tubulin was not detected, it has been shown that the S409 phosphorylation inhibits binding of tau from yeast extracts to mammalian tubulin (Vandebroek et al., 2006). This confirms an antagonistic relationship between the abilities of tau to bind microtubules and to form aggregates. Oxidation stress (induced by Fe^{2+}) or mitochondrial dysfunction (due to mutations) are shown to enhance tau aggregation in yeast (Vanhelmont et al., 2010). While tau was not toxic on its own to the wild-type or *pho85Δ* yeast strains, it became toxic in the strain with defective Ess1, as per preliminary data published in the review paper (De Vos et al., 2011), or when coexpressed with α-synuclein (Ciaccioli, Martins, Rodrigues, Vieira, & Calado, 2013), known to promote tau aggregation from mammalian studies, *e.g.* (Riedel, Goldbaum, & Richter-Landsberg, 2009).

In addition to the expression of tau in the cytosol, the model has been developed in which tau is displayed on surface of the yeast cells (Wang & Cho, 2019). While being applicable to studying interactions between tau and other proteins (including antibodies, recognizing filaments of aggregated tau), this model does not address tau phosphorylation and nucleation of tau aggregates.

Overall, yeast studies produced some data that are important for understanding the relationship between tau hyperphosphorylation, aggregation and microtubule binding. However, it still has to be determined if tau aggregates formed in yeast are of the same type as those associated with human diseases, and convenient phenotypic or cytological assays for tau aggregation in yeast are still lacking, that prevents efficient searches for agents counteracting tau aggregation and/or toxicity.

6. Yeast models for aggregation of mammalian prion protein (PrP)

Prion protein (PrP) in its “scrapie” or prion form (PrP^{Sc}) is a causative agent of TSEs, or prion diseases, which are fatal incurable neurodegenerative disorders found in various mammals, including goat and sheep (scrapie), cattle (bovine spongiform encephalopathy or “mad cow” disease), elk, deer and moose (chronic wasting disease), some predators (*e.g.* mink encephalopathy) and humans (kuru, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia) (Colby & Prusiner, 2011; Prusiner, 1998, 2013). In addition to heritable (about 15% of patients in humans that are all carrying mutations in the PrP-coding gene) and sporadic cases, TSEs can also be infectious. While relatively rare in humans, TSEs attracted a lot of attention due to documented cases of BSE transmission to humans (Collinge, 1997; Prusiner, 1998). PrP^{Sc} is a cross-β polymer that produces amyloid deposits in patient’s brains and *in vitro.* Rodents (especially mouse and hamster) are frequently used as experimental models for studying TSEs. The major obstacle to studying molecular basis of TSEs are long incubation periods, than take several years in cows and humans, and months even in rodents (Colby & Prusiner, 2011). Normal cellular

function of PrP remains elusive, despite many years of studies. At least, TSE disease is not a consequence of the loss of PrP function, because mice homozygous by the deletion of PrP-coding gene do not develop disease symptoms but rather are resistant to the infection by PrP^{Sc} (Weissmann & Flechsig, 2003).

Structural and functional organization of mouse PrP, used in the majority of yeast studies, is shown in Fig. 8A, which is based on (Flechsig & Weissmann, 2004; Prusiner, 1998, 2013) and other studies reviewed there. The cellular (non-prion) form of PrP (PrP^{C}) is a glycosylated protein, which is secreted (using the N-terminal signal peptide between aa positions 1 and 22, that is processed out during secretion) and attached to the outer surface of the plasma membrane with a glycophosphatidylinositol (GPI) anchor, using the C-terminal region of PrP, located between the aa positions 231 and 254 (Stahl, Borchelt, Hsiao, & Prusiner, 1987). Neither PrP glycosylation nor GPI anchor appear to be required for the PrP conversion into a prion form, and for infectivity. Therefore, some studies employ the 23—230 PrP fragment. Moreover, the intrinsically disordered 23–89 region is not included into an amyloid core and is dispensable for the PrP-mediated infection, although this region contains five oligopeptide (octapeptide) repeats of consensus sequence P(Q/H)GGG(G/–)WGQ, somewhat similar (although not homologous) to the oligopeptide repeats of the yeast Sup35 protein (shown in Fig. 2 above), and mutations leading to expansions of these repeats are shown to be associated with heritable prion disease in humans. In contrast, the region encompassing aa positions 90 and 119, which is also overlapping a portion of the intrinsically disordered N-proximal segment of PrP^{C} (Fig. 8A), is required for the susceptibility to TSE in mammalian systems (Flechsig & Weissmann, 2004).

In order to reproduce PrP secretion patterns in yeast, the hydrophobic core of the signal peptide of mouse PrP was replaced by the signal sequence from the yeast excreted protein dipeptidyl aminopeptidase B, so that the resulting protein would be targeted cotranslationally to the yeast secretory pathway. PrP molecules with the modified signal peptide were efficiently secreted, glycosylated, glycolipid-anchored, and localized to the plasma membrane (Li, Dong, & Harris, 2004). This model was used to show that PrP^{C} is unlikely to play a direct role in trafficking of Cu^{2+} or Zn^{2+} ions, as it was proposed earlier. It can also potentially be useful for identification of proteins interacting with PrP^{C}. However, it is not clear how PrP polymerization into PrP^{Sc} is to be monitored in this model.

Unglycosylated mouse PrP lacking a signal peptide and expressed in yeast forms fibrous cytosolic aggregates, which are protease-resistant and detergent-insoluble, as typical of PrP^{Sc} (Ma & Lindquist, 1999). While most PrP is extracellular in mammalian cells, it is not yet clear where the prion isoform (PrP^{Sc}) is initially formed, so that the authors proposed that PrP could undergo aggregation in result of retrograde transport from the ER/secretory pathway to the cytosol. The PrP aggregates formed in yeast cells are able to convert normal PrP^{C} from the mouse brain homogenates into a proteinase K-resistant conformation, resembling PrP^{Sc} (Yang, Yang, & Tien, 2006). PrP or its derivatives encompassing the 90–230 region produced detergent-resistant aggregates detectable by FM in yeast cytoplasm when they were fused to the fluorophore, such as yellow (YFP), cyan (CFP) or green (GFP) fluorescent protein as shown in Fig. 8B (Rubel et al., 2013, 2008). In addition, PrP

aggregates formed in yeast interacted with amyloid-specific aptamers (Mitkevich et al., 2012). These results indicate that PrP, produced in the cytosol of yeast cells, possesses biochemical properties that are similar to disease-associated PrP^{Sc} from the brains of sick animals. However, infection of animals by aggregated PrP from yeast cells has never been reported. This has to be noted that the infection of animals with recombinant PrP produced *in vitro* is also inefficient, and some positive results have been achieved relatively recently as reviewed in (Charco et al., 2017; Legname & Moda, 2017).

In contrast to mammalian neurons, PrP aggregates are not toxic to yeast and do not affect the growth of yeast cultures. However, deletions of some genes associated with proteasome system inhibit the growth of strains expressing PrP (Apodaca, Kim, & Rao, 2006). It is not yet clear if this effect is related to PrP aggregation.

This has also been demonstrated that the interaction between the PrP protein with a methionine residue at the position 129 (PrP-129M), and the PrP protein with valine at this position (129V) is reduced in the yeast cells, compared to homotypic combinations (Mallik, Yang, Norstrom, & Mastrianni, 2010). Position 129 is polymorphic in humans, and this has been shown that the 129M/129V heterozygotes are less susceptible to prion disease and infection (Kobayashi, Hizume, Teruya, Mohri, & Kitamoto, 2009; Mead et al., 2008; Nystrom & Hammarstrom, 2014), that agrees with the less efficient interaction between heterotypic PrP molecules. Importantly, an introduction of the substitution P101L (that is equivalent to the disease-associated substitution P102L in human PrP) into a fluorophore-tagged mouse PrP abolished fluorescence resonance energy transfer (FRET) with wild-type PrP in yeast, whereas mutant PrP-P101L displayed high FRET with identical PrP-P101L, as long as residue 129 was matched (Mallik et al., 2010).

Fusions of mouse PrP or PrP-derived fragments to fluorophores allow for the analysis of protein-protein interactions that involve aggregated PrP (Fig. 8B). By using FRET, it has been demonstrated that aggregated fluorophore-tagged PrP and Aβ proteins physically interact in yeast cells (Rubel et al., 2013). PrP sequences essential for such an interaction have been identified. This agrees with previous reports on Aβ-PrP interactions from mammalian systems, *e.g.* (Chen, Yadav, & Surewicz, 2010). However, mammalian studies typically targeted PrP^{C}, thus the ability of aggregated PrP to interact with Aβ represents a contribution of the yeast model.

Another approach for studying PrP aggregation in yeast (Josse, Marchante, Zenthon, von der Haar, & Tuite, 2012) employed the yeast prion-forming Sup35 protein as a reporter, in a manner similar to that described above for the Aβ-MRF fusion (Park et al., 2011). In the yeast model for PrP (Fig. 8C), most of the N-terminal domain of Sup35 (up to the position 97) was replaced with either the core region of mouse PrP (aa positions 88–240) or the longer region (49–240) including oligopeptide repeats (49–240) (Josse et al., 2012). These constructs are designated here and further as PrP-MRF, to keep in line with Aβ-based constructs, although authors used different nomenclature. In addition, authors constructed a chimera in which only the NQ stretch of Sup35 was replaced by the 49–240 PrP region (designated here and further as PrP-NR-MRF). These chimeric constructs were expressed in yeast cells, lacking the endogenous *SUP35* gene. The PrP-MRF constructs (with or without

PrP repeats) aggregated in yeast, but these aggregates were not resistant to sodium dodecyl sulfate (SDS). In contrast, the chimera, in which in only the N-terminal QN-rich stretch of Sup35 PrD has been substituted by PrP and both the PrP and Sup35 repeat regions were present, formed SDS-resistant polymers. This should be noted that according to our preliminary results, aggregates formed by PrP-Sup35 constructs similar to those employed by Josse et al. (2012) are resistant to sarkosyl (A. Rubel, Y. Chernoff, A. Galkin, unpublished data), and in case of PrP-NR-MRF, this resistance does not depend on the presence or absence of the oligopeptide region of PrP (V. Lashkul, D. Kachkin, Y. Chernoff, A. Rubel, unpublished data). Apparently, the combination of the oligopeptide regions of both PrP and Sup35 is needed specifically for resistance to SDS, rather than for the formation of the detergent-resistant fibrils in general.

Phenotypic analysis of the yeast strains producing various chimeric constructs has shown that attachment of PrP leads to a decrease in the termination function of Sup35, thus resulting in translational readthrough and nonsense-suppression (similar to what is described above for Aβ-MRF). The presence of oligopeptide repeats from mouse in the PrP-NR-MRF chimera enhanced suppression, although the replacement of the Sup35 repeat region by the repeat region from PrP (see construct designated as Sup35QN-PrP(49–90)-MRF on Fig. 8C) did not inhibit Sup35 function if other PrP-derived sequences were not present. It is not clear if nonsense-suppression was a consequence of aggregation or caused by other reasons, such as interference of the PrP-derived sequence with the folding of the globular domain and/or proper functioning of Sup35. However, the readthrough-stimulating effect of the addition of the repeat region of PrP (which also increases the detergent-resistance of aggregates) is a strong indication to that aggregation contributes to this phenotype. Notably, neither overproduction nor inactivation of the Hsp104 chaperone, known to control the propagation of the Sup35 prion (see above), affected the nonsense suppressor phenotype in strains expressing the PrP-Sup35MC constructs. This points to the differences between yeast prions and PrP-mediated aggregates in yeast. Likewise, quinacrine, a compound which has been reported to efficiently inhibit PrP polymerization and PrP^{Sc} accumulation in cultured cells and some other previously tested compounds with anti-PrP activity did not impact the PrP-mediated nonsense-suppression in yeast, pointing to the differences between the aggregates formed by PrP-based chimeras in yeast, and PrP prions in native environments (Josse et al., 2012). It is worth noting that quinacrine can antagonize the endogenous yeast prion [PSI^{+}] formed by Sup35 protein, and some other compounds initially identified by their anti-[PSI^{+}] effect were shown to antagonize PrP^{Sc} in cultured mammalian cells (Bach et al., 2003; Nguyen et al., 2014; Voisset et al., 2017). Therefore, endogenous [PSI^{+}] prion recapitulates some aspects of PrP propagation better than some artificial PrP-based chimeric constructs.

While PrP-based assays developed in yeast produced some interesting data, convenient yeast model for tracking the PrP prion propagation that would be relevant to mammalian systems is still lacking. However, data described in Section 9 below show that the processes leading to the initial nucleation of PrP polymers can be modeled in yeast.

7. Yeast models for proteins associated with amyotrophic lateral sclerosis (ALS)

7.1 Overview of ALS-associated proteins

ALS is the progressive neurodegenerative disease that affects upper and lower motor neurons, and is characterized by a rapidly progressive loss of motor neurons in the spinal cord, muscle weakness and paralysis. In many cases, ALS is associated with accumulation of aggregated proteins in the form of cytoplasmic inclusion in central neurons and glial cells. These inclusions may contain trans-activation response element (TAR) DNA-binding protein 43 (TDP-43) and some others RNA-binding proteins (FUS, SOD1, hnRNPA2, TAF15, EWS). Some of the same proteins have also been detected in aggregated form in patients with frontotemporal dementia (FTD, see above), suggesting that some forms of ALS and FTD may have a common cause (Couratier, Corcia, Lautrette, Nicol, & Marin, 2017). Approximately 90% of the ALS cases are non-heritable (that is, sporadic), while remaining 10% are heritable, or familial (fALS). Among a growing number of genes associated with fALS, mutations in four genes are most common: *C9orf72* (~ 40% of heritable cases), *SOD1* (~20%), *FUS* (~1–5%), and *TARBDP* (~1–5%). Other rare gene mutations associated with fALS include mutations in *UBQLN2* (ubiquilin 2), *OPTN* (optineurin), *VCP* (valosin-containing protein) and *TBK1* (TANK-binding kinase 1) (Renton, Chio, & Traynor, 2014).

Yeast models are successfully applied to elucidation of the molecular mechanisms underlying the development of ALS pathologies. Among the proteins associated with ALS, TDP-43 and FUS are most extensively studied in yeast (Di Gregorio & Duennwald, 2018; Kryndushkin, Wickner, & Shewmaker, 2011; Lindstrom & Liu, 2018; Monahan, Rhoads, Yee, & Shewmaker, 2018).

7.2 TDP-43

TDP-43 is highly conserved RNA/DNA-binding protein involved in RNA processing, including splicing, transcription and transport. TDP-43 is expressed in a variety of cell types and is predominantly localized in the nucleus, but also performs some functions in the cytoplasm (Ayala et al., 2008). TDP-43 protein (Fig. 9A) includes the following important regions: (1)N-terminal domain (aa 1–102) that mediates self-assembly and contains a nuclear localization signal (NLS, aa 82–98); (2) two RNA recognition motifs, RRM1 (aa 104–176) and RRM2 (aa 192–262); (3) a nuclear export signal (NES, aa 239–250); and (4) an intrinsically disordered low complexity domain (LCD) at the C-terminus (aa 274–414), that includes a QN-rich domain (aa 345–366) and a G-rich stretch (aa 366–414) (Jiang et al., 2016; Kuo, Chiang, Wang, Doudeva, & Yuan, 2014; Mompean et al., 2016; Qin, Lim, Wei, & Song, 2014). The aa composition of the QN-rich domain of TDP-43 is similar to that of yeast prion domains, therefore this portion of the protein is termed a “prion domain like domain” (PrLD) region.

Majority of the sporadic ALS cases are associated with the deposition of the TDP-43 protein, phosphorylated at S409 and S410 positions, and its 25 and 35 kD C-terminal cleavage fragments in neuronal and glial cytoplasmic inclusions (Mackenzie et al., 2007; Neumann et al., 2006). This points to a key role of TDP-43 aggregation in ALS pathology.

Moreover, mutations in TDP-43 (mostly within its G-rich C-terminal domain) are associated with about 5% of fALS cases (Gendron et al., 2013; Sreedharan et al., 2008). LCD is responsible for liquid-liquid phase separation and inclusion of TDP-43 into stress granules (SGs). Disease-linked TDP-43 mutations lead to excessive accumulation of SGs in cells and its reduction in the cell nucleus (Johnson et al., 2009; Nonaka, Kametani, Arai, Akiyama, & Hasegawa, 2009).

It is still debated whether or not TDP-43 inclusions, detected in the neuronal cells possess amyloid properties. It has been reported that neither filamentous TDP-43 assemblies, found in the ALS-affected brains, nor aggregates formed by recombinant TDP-43 *in vitro* or in *Escherichia coli* cells are stained with thioflavin-T (ThT) or Congo red (CR), dyes that are routinely used to detect cross-β amyloid structures (Capitini et al., 2014; Johnson et al., 2009; Neumann et al., 2006). However, some other studies point to the formation of β-sheet-rich, ThT-positive fibrillar aggregates, similar to amyloids by both wild-type TDP-43 and its derivative, bearing an ALS-associated mutation (Bigio et al., 2013; Robinson et al., 2013; Zhu et al., 2014). In addition, prion-like spread of TDP-43 aggregates has been demonstrated in mammalian cell models (Feiler et al., 2015; Furukawa, Kaneko, Watanabe, Yamanaka, & Nukina, 2011; Ishii, Kawakami, Endo, Misawa, & Watabe, 2017; Nonaka & Hasegawa, 2018; Smethurst et al., 2016).

Yeast studies made a significant contribution to current understanding of the pathogenicity of TDP-43 and were mostly confirmed by using other approaches (Di Gregorio & Duennwald, 2018; Lindstrom & Liu, 2018; Monahan et al., 2018). It was shown that the yeast model for TDP-43 recapitulates its key pathology-relevant features seen in humans, including normal nuclear localization and cytotoxic cytoplasmic sequestration (Johnson, McCaffery, Lindquist, & Gitler, 2008). Notably, the C-terminal LCD region (including PrLD) was implicated as one playing a critical role in TDP-43 aggregation (Johnson et al., 2008). Most ALS-associated mutations increased TDP-43 aggregation and toxicity in yeast, although some mutations having no such effect were also reported (Johnson et al., 2009). Mitochondrial function and oxidative stress were also linked to the TDP-43-triggered toxicity in yeast (Braun et al., 2011). Indeed, TDP-43 toxicity is enhanced in respirating yeast cells, although TDP-43 remains toxic even in the absence of respiration (Park, Park, & Liebman, 2019). Random mutagenesis of the PrLD-coding region revealed that mutations increasing TDP-43 toxicity typically promote phase separation (that is, formation of the dynamic liquid-like cytoplasmic condensates), while the increase in hydrophobicity and aggregation *per se* reduces toxicity (Bolognesi et al., 2019).

Yeast model has also been effectively used as a tool for identification of the proteins that influence TDP-43 aggregation and toxicity. By screening the yeast deletion library, it was shown that expression of TDP-43 is not toxic to the yeast cells lacking the *DBR1* gene, that codes for the protein involved in splicing (Armakola et al., 2012; Daigle et al., 2013; Figley & Gitler, 2013). This was confirmed by showing that knocking down of the *DBR1* homolog in human cells also protects against TDP-43 cytotoxicity (Armakola et al., 2012). Possibly, splicing intermediates accumulated in cytosol in the absence of Dbr1 sequester TDP-43, preventing it from interfering with essential RNAs and/or RNA-binding proteins.

Additional modifiers of TDP-43 toxicity revealed by high-throughput screens in yeast include RNA-binding proteins such as poly-A binding protein Pab1 and a yeast ortholog of the translation initiation factor EIF2A, that are incorporated into SGs during stress; these findings were confirmed in *Drosophila melanogaster* model (Kim et al., 2014).

Engineered "potentiated" (hyperfunctional) derivatives of yeast chaperone Hsp104 (see above) rescued cytotoxicity of TDP-43, as well as that of another ALS-associated protein, FUS (described below) and of αSyn in yeast (Jackrel et al., 2014; Jackrel & Shorter, 2014). While Hsp104 is not present in mammals, the engineered Hsp104-based constructs can potentially be introduced there as an agent, counteracting disease, although the potential side effects on other processes should be of course characterized first. Overexpression of the Hsp40 chaperone Sis1 also antagonized toxicity of both TDP-43 and FUS in the yeast model, that agrees with the effect of this protein on polyQ toxicity (see above) and is confirmed by studying the impact of its human ortholog DnaJB1 on TDP-43 and FUS toxicity in mammalian and human cell models, including and primary cortical neurons (Park et al., 2018, 2017). The yeast studies also demonstrated that the endosomal-vacuolar trafficking pathway and the vacuolar (lysosomal) protein degradation machinery are crucial for the TDP-43 degradation and cell survival in yeast, although autophagy increased toxicity, indicating a complex impact of the lysosome-associated pathways on the TDP-43 associated (Leibiger et al., 2018).

7.3 FUS

Another protein associated with ALS, whose role in this disease is largely understood from the yeast models (Di Gregorio & Duennwald, 2018; Lindstrom & Liu, 2018; Monahan et al., 2018) is a FUS protein, coded by the gene *FUS/TLS.* The name of this gene is coming from "fused in sarcoma/translocated in sarcoma" and has no relation to its function. Like TDP-43, FUS is a RNA/DNA-binding protein that plays a role in numerous cellular processes, including transcription, splicing, microRNA maturation, RNA transport and SG formation, and can shuttle between the nucleus and cytosol. Normally, FUS is predominantly nuclear in glial cells and neurons. Relocalization of FUS to cytosolic aggregates and the decrease in the proportion of the nuclear FUS fraction is an important hallmark of some ALS cases (Lagier-Tourenne & Cleveland, 2009). Mutations in FUS cause around 5% of all familial ALS cases, with disease phenotypes inherited in an autosomal dominant fashion (Renton et al., 2014). Interestingly, TDP-43 aggregation is not detected in fALS cases with FUS mutations (Vance et al., 2009).

FUS protein (Fig. 9B) contains the N-terminal PrLD or LCD (Q/G/S/Y-rich) domain (aa positions 1–165), the Gly-rich region (aa 166–267), and the C-terminal region includes RRM (aa 285–371), two RGG-repeat regions (aa 371–422 and 453–501), a zinc finger motif, or ZNF (aa 422–453), and a non-conventional nuclear localization signal, NLS (aa 510–526) (Dormann et al., 2012, 2010; Iko et al., 2004). Most of the fALS-associated FUS mutations are located in the N-terminal LCD domain, in the second RGG domain or with NLS in the C-terminal region. Disease-associated mutations accelerate the phase transition of FUS, promoting sequestration of wild-type FUS protein into SGs, and delaying SG formation in response to stress, while accelerating SG dissociation (Murakami et al., 2015;

Patel et al., 2015). These data suggest direct pathological role of SGs in ALS. Normal function of FUS is not critical for the survival of motor neurons, suggesting that neurodegeneration is a consequence of a gain of toxicity (Sharma et al., 2016), although some researchers still dispute this notion, *e.g.* (Lindstrom & Liu, 2018). Abnormal FUS phase transition has been suggested to be a causative factor in ALS (Hofweber et al., 2018; Luo et al., 2018; Murray et al., 2017; Qamar et al., 2018). While yeast does not have a FUS ortholog, many cell pathways in which FUS is involved are conserved in yeast (Ju et al., 2011). Several yeast models that express human FUS, either in wild-type form or with ALS-linked mutations have been introduced and exploited, as described below.

Yeast models served as a powerful tool for identifying the FUS domains, that are responsible for its nuclear versus cytoplasmic localization and aggregation. In contrast to mammalian cells, not only mutant FUS but also a full-length wild-type FUS protein are preferentially localized in the cytosol and forms numerous cytoplasmic aggregates in yeast cells (Ju et al., 2011). This difference suggests that the non-canonical NLS of FUS is insufficient to efficient localize protein to the nucleus in yeast (Ju et al., 2011). Addition of strong heterologous SV40 NLS to FUS increased its localization to the nucleus and eliminated cytoplasmic aggregation of FUS. Thus, defective nuclear import of FUS might be a key upstream event in ALS (Sun et al., 2011). Moreover, FUS lacking the RGG regions localized to the nucleus even if it did not contain NLS, while addition of one of the RGG regions restored its cytosolic localization in yeast (Sun et al., 2011). Apparently, interaction with RNA via RGG domain is crucial for the retention of FUS in the cytosol. It was also shown that PrLD is required for the aggregation of FUS in the cytoplasm (Sun et al., 2011). Respective roles of these domains were later confirmed using insect and human cell models (Patel et al., 2015; Sun et al., 2011).

Yeast studies have demonstrated that FUS toxicity to the yeast cells is increased with the increase of its expression levels, and is associated with FUS accumulation in cytosolic aggregates (Kryndushkin et al., 2011). Despite similarities between FUS and TDP-43 proteins, they apparently aggregate in yeast via distinct mechanisms, as in addition to the PrLD region of FUS, RRM and RGG regions are also required for toxicity and the C-terminal region is involved (Kryndushkin et al., 2011; Sun et al., 2011). As FUS PrLD is extensively phosphorylated during stress in human cells (Rhoads et al., 2018), phosphomimetic substitutions were introduced into FUS and shown to reduce aggregation and ameliorate FUS-associated cytotoxicity in both human cell and yeast models (Monahan et al., 2017).

A genome-wide genetic screen using a yeast overexpression library demonstrated that the proteins involved in SG assembly and RNA metabolism modify FUS toxicity (Ju et al., 2011; Sun et al., 2011). Effect of one of the antagonists of FUS toxicity, yeast RNA helicase Ecm32, was confirmed for its human homolog (Daigle et al., 2013; Ju et al., 2011; Sun et al., 2011). Yeast two-hybrid screens identified protein arginine methyltransferase 1 (PRMT1) as one of binding partners of FUS (Yamaguchi & Kitajo, 2012). Methylation of FUS by PRMTs has subsequently been shown to be important for regulation of its nuclear versus cytoplasmic localization (Tradewell et al., 2012).

Despite the obvious similarity between FUS PrLD and PrDs of most yeast prion proteins, it is still unclear whether or not FUS assemblies, associated with ALS possess amyloid properties (Cushman, Johnson, King, Gitler, & Shorter, 2010; Udan & Baloh, 2011). In contrast to typical amyloids, FUS aggregates formed in yeast cells were initially reported to be detergent-sensitive (Ju et al., 2011; Kryndushkin et al., 2011), while aggregates formed by recombinant FUS *in vitro* are not stained by ThT, usually staining amyloid fibrils (Sun et al., 2011). However, another study described ThT staining of FUS aggregates in yeast, as well as their resistance to a mild detergent, 0.5% sarkosyl (Fushimi et al., 2011). Mutant LCD region of FUS can form fibrils *in vitro* that are similar to typical amyloid fibrils by morphology (Zhu et al., 2014). However, no matter what is the molecular basis of FUS aggregation, it possesses at least one unique feature: in contrast to amyloid-based prions and most disease-associated amyloids, FUS aggregation is reversible and can be regulated. FUS amyloid fibrils can be disassembled depending on FUS concentration, phosphorylation of the LCD domain, and DNA or RNA levels (Han et al., 2012; Kato et al., 2012; Kwon et al., 2013; Schwartz, Wang, Podell, & Cech, 2013).

7.4 Other ALS-associated proteins

In addition to TDP-43 and FUS, about 20 genes/proteins have been linked to ALS at present. For some of them, studies in the yeast models have also been performed.

7.4.1 C9orf72—A massive GGGGCC hexanucleotide repeat expansion in the first intron of the *C9orf72* gene has been identified as one of the most frequent causes for fALS and FTD, being linked to about 40% cases of heritable ALS (Renton et al., 2014). The following (mutually non-exclusive) mechanisms for this expansion were proposed.

1. Decrease in the levels of functional C9orf72 protein due to interference of the expansion with gene expression (DeJesus-Hernandez et al., 2011; Waite et al., 2014).
2. Formation of toxic secondary RNA structures (that sequester RNA-binding proteins) due to bidirectional transcription of the expanded repeat region (DeJesus-Hernandez et al., 2011; Gendron et al., 2013; Haeusler et al., 2014).
3. Non-AUG initiated translation of sense and antisense repeat-containing RNAs, producing aggregation-prone polypeptides with dipeptide repeats (DPR), such as GP, GA, GR, PA and PR. Of these, PR and GR containing polypeptides are most toxic in a variety of model systems, ranging from human cells to yeast (Ash et al., 2013; Mori et al., 2013; Zu et al., 2013). A genetic screen for modifiers of PR and GR toxicity identified toxicity suppressors and enhancers and pointed to that toxicity of PR and GR containing polypeptides is possibly due to distinct mechanisms (Chai & Gitler, 2018; Jovicic et al., 2015).

7.4.2 Sod1—Up to 20% of fALS cases are associated with an over 150 mutations within the gene encoding Cu,Zn-superoxide dismutase 1 (Sod1), a primarily cytosolic homodimeric protein implicated in the defense against free radicals (Renton et al., 2014). Wild-type Sod1 protein also can participate in the ALS pathology, including some cases associated with other proteins (Graffmo et al., 2013), and aggregates of TDP-43 or FUS can promote

misfolding of wild-type Sod1 in cultured cells (Pokrishevsky, Grad, & Cashman, 2016). In general, misfolded and/or unstable Sod1 protein is widely reported as a hallmark of ALS. It is still not fully understood how is Sod1 misfolding related to cellular dysfunction. Interestingly, recent studies have shown that misfolded toxic aggregates of mutant (Ayers, Fromholt, O'Neal, Diamond, & Borchelt, 2016; Munch & Bertolotti, 2011; Munch, O'Brien, & Bertolotti, 2011; Pokrishevsky, Hong, Mackenzie, & Cashman, 2017) or wild-type (Grad & Cashman, 2014; Grad et al., 2014; McAlary, Plotkin, Yerbury, & Cashman, 2019) Sod1 exhibit prion-like properties so that the misfolded state of the protein can be transmitted between cells, although amyloid formation *per se* has not been reported.

The *S. cerevisiae* yeast possesses the Sod1 ortholog (with the same name) showing 54% aa identity with human Sod1. Wild-type or mutant human Sod1 are enzymatically active in yeast and restore the wild-type phenotype in the *sod1Δ* yeast strain (Rabizadeh et al., 1995). This suggested that ALS-associated mutations in *SOD1* gene do not influence the functional activity of the Sod1 protein. This was further confirmed by using a transgenic mice model with overexpressed human SOD1 mutants (Reaume et al., 1996). Yeast studies demonstrated that the accumulation of some fraction of wild-type Sod1 in the mitochondrial intermembrane space (IMS), mediated by the copper chaperone, CCS is protecting cells against oxidative stress (Sturtz, Diekert, Jensen, Lill, & Culotta, 2001). One of the common ALS-linked mutations in Sod1 (G93A) leads to an increased Sod1 accumulation in IMS and (somewhat surprisingly) increases the protection of yeast cells from mitochondria-induced oxidative stress (Kloppel, Michels, Zimmer, Herrmann, & Riemer, 2010). Such an effect was not detected for the catalytically inactive (G85R) Sod1 mutant. Defects in the assembly of the electron transport complex after expression of some mutant derivatives of Sod1 in yeast have also been reported (Gunther, Vangilder, Fang, & Beattie, 2004). Other data point to the correlation of the toxic effect of some Sod1 with the decrease in vacuole acidification, that antagonizes vacuolar proteolysis, perturbs metabolic regulation and promotes senescence (Bastow et al., 2016). It is possible that due to a high pleiotropy of superoxide dismutases, different mutations may influence cell viability and lead to disease via different mechanisms.

7.4.3 HnRNP—The hnRNP (heterologous nuclear ribonucleoprotein) family includes paralogous RNA-binding proteins, among them hnRNPA1 and hnRNPA2B1, that is represented by isoforms A2 and B1, originated from alternative splicing (Kim et al., 2013). Each of these proteins possesses a G-rich PrLD (see Fig. 9C for hnRNPA2B1), as predicted by sequence analysis using an algorithm derived from studying yeast prions. Mutations found in the conserved PrLD region of hnRNPA1 and hnRNPA2B1 are associated with an unusual disease, that combines features of ALS and some other disorders (inclusion body myopathy, FTD and Paget's disease of bone), also involves aggregation of TDP-43 and is sometimes termed "multisystem proteinopathy," or MSP (Kim et al., 2013). Notably, hnRNPA1 and hnRNPA2B1 are known to interact with TDP-43, and hnRNPA2B1 has been implicated in another neurological disease, fragile X-associated tremor ataxia syndrome (FXTAS), where it binds expanded rCGG repeats, underlying this disease (Iwahashi et al., 2006; Sofola et al., 2007).

The system employed for studying hnRNPA2B1 in yeast (Kim et al., 2013; Paul et al., 2017) employed a chimeric construct with Sup35, termed A2-Sup35 (Fig. 9D) and based on the

principle similar to one described above for PrP. The region encompassing the first 40 aa (that includes the QN-rich aggregation-prone stretch of PrD) of the Sup35 protein has been substituted by the PrLD region (aa residues 261—303) from hnRNPA2. In contrast to the PrP-based construct, this chimeric protein was functional in translation termination, leading to the Ade^- phenotype in the reporter yeast strain (in this case, containing the *ade2-1* UAA allele). However, spontaneous Ade^+ colonies appeared at the frequency of about 10^{-4}. The frequency of Ade^+ formation was increased by transient overexpression of the hnRNPA2B1 PrLD region fused to GFP (A2-PrLD-GFP) in the same cells. The prion nature of these Ade^+ colonies was confirmed by demon-strating that most of them were "cured" (that is, reverted to Ade^-) after the growth in the medium containing guanidine hydrochloride, a compound that inhibits Hsp104 and is known to "cure" the Hsp104-dependent yeast prions, including [*PSI*$^+$], as reviewed in (Chernova et al., 2014). Aggregation of the A2-Sup35 protein in Ade^+ cells was visualized by fluorescence microscopy after co-expression of the A2-PrLD-GFP construct (Paul et al., 2017). Authors do not specifically address whether or not the yeast strain used in this work contains other pre-existing prions; data reported in their work are consistent with the presence of prion [*PIN*$^+$] that promotes aggregation of other prion proteins in yeast.

By using this system, authors studied effects of various amino acid substitutions at the disease-associated aa position 290 of hnRNPA2B1 protein on the ability of the respective A2-Sup35 chimeric construct to convert into a prion state in yeast (Paul et al., 2017). Results were compared to the predictions made by PAPA bioinformatic algorithms developed by the same group, that assesses prion propensity of various amino acid residues in yeast (Shattuck, Waechter, & Ross, 2017; Toombs et al., 2012). While the PAPA algorithm was able to accurately predict prion propensities of the mutations in the hnRNPA2B1 PrLD in yeast and *in vitro,* some mutations did show different results in the *Drosophila* model for the aggregation of full-length hnRNPA2B1, indicating that either yeast-derived sequences (used in a chimeric construct) or cellular environment makes certain impact on the ability to propagate the prion state dependent on the hnRNPA2B1 PrLD region (Paul et al., 2017). The difference in prion propagating machineries could be expected due to absence of Hsp104 chaperone in the animal cells, and this could be an obstacle for other yeast assays based on the ability to propagate heterologous prions as well. In the subsequent paper (Cascarina, Paul, Machihara, & Ross, 2018), authors employed the same yeast model for comparing effects of aa composition of the hnRNPA1 and hnRNA2B1 PrLD regions, and of Sup35 PrD on the balance between aggregation and degradation propensities. They have shown that large aliphatic residues that are known to enhance aggregation in other systems actually promote degradation of the G-rich PrLD regions in yeast, whereas aromatic residues enhanced aggregation without promoting degradation. Interestingly, the degradation-promoting effect of aliphatic residues was suppressed in the context of the Q/N-rich prion domain, that may explain QN-richness of yeast PrDs, and of many PrLD regions in other proteomes. This remains to be seen if these findings are applicable to human cells.

7.4.4 Other proteins associated with ALS—Yeast models also helped to investigate the mechanisms of toxicity caused by some rare ALS-associated mutations in the following proteins: VAPB (Nakamichi, Yamanaka, Suzuki, Watanabe, & Kagiwada, 2011); VCP

(Takata et al., 2012); OPTN (Kryndushkin, Ihrke, Piermartiri, & Shewmaker, 2012); SETX (Bennett & La Spada, 2018; Richard, Feng, & Manley, 2013); profilin 1 (Figley, Bieri, Kolaitis, Taylor, & Gitler, 2014). Recently, a yeast model was used for characterization of the toxicity of calcium-responsive transactivator (CREST), chromatin-remodeling protein whose mutant variant is associated with some cases of ALS (Chesi et al., 2013; Teyssou et al., 2014). CREST contains QN-rich PrLD and forms toxic nuclear (and occasionally cytoplasmic) aggregates stained by ThT in the yeast cells (Park, Park, Watanabe, et al., 2019). Toxicity of CREST to yeast cells is enhanced by the presence of the QN-rich prion protein Rnq1 in a prion form, and reduced by the deletion of a gene coding for the chaperone Hsp104, required for the fragmentation and propagation of most known endogenous yeast prions (see above). Deletion of the *PBP1* gene, coding for the yeast ortholog of one of the human ataxin proteins (ATXN2) reduced aggregation and toxicity of CREST in yeast cells. Notably, ATXN2 itself contains the polyQ PrLD region, and intermediate expansion (up to 27—34 residues) of the ATXN2 polyQ tract is a known risk factor associated with FTD and ~5% of ALS cases (Becker et al., 2017). Moreover, CREST and PBP1/ATXN2 co-localize in both yeast and mammalian cells. These observations were also confirmed in a transgenic *Drosophila* model for CREST (Park, Park, Watanabe, et al., 2019).

8. Aggregation of transthyretin in yeast

Transthyretin (TTR) is a protein associated with amyloidosis affecting various tissues, including transthyretin amyloidosis (ATTR) and familial polyneuropathy (Connors, Lim, Prokaeva, Roskens, & Costello, 2003). Most cases of TTR-associated amyloidoses are heritable and caused by mutations in TTR. Native TTR is a tetramer; some amyloid-associated mutations disrupt the tetramer assembly and promote amyloid formation as an alternative assembly pathway (Johnson, Connelly, Fearns, Powers, & Kelly, 2012). However, non-mutant TTR can also aggregate in systemic amyloidosis (Westermark, Engstrom, Johnson, Westermark, & Betsholtz, 1990).

It has been shown that TTR with a disease-promoting mutation (TTR-L55P) forms detergent-insoluble aggregates in yeast cells, while wild-type TTR does not (Gomes et al., 2012). Analysis of the changes in the yeast proteome composition, occurring in response to TTR-L55P aggregation reveled increased abundance of the following proteins: some enzymes of the tricarbon acid cycle, involved in respiration; superoxide dismutase; some chaperones of the Hsp70 family (specifically, Ssa1 and Ssa2); the ubiquitin-like protein Smt3 (yeast ortholog of mammalian SUMO, previously linked to AD and HD); and members of the peptidyl-prolyl-*cis-trans* isomerase (PPIase) family (cyclophilin A and FKBP), whose human homologs were identified as TTR-interacting partners in ATTR patients. Overall, these data are consistent with that aggregation of mutant TR leads to the increased mitochondrial respiration, promoting oxidative stress, that is likely to be relevant to the mechanism of pathogenicity of TTR amyloids in humans.

Another work has shown that the mutant (M) TTR-GFP construct, bearing double amino acid substitution at positions, associated with TTR amyloidoses (F87M/L110M) formed insoluble aggregates, detectable by fluorescence microscopy in yeast cells at acidic pH

(Verma et al., 2018). Aggregation of M-TTR-GFP was enhanced in the presence of the Sup35 prion, [*PSI*$^+$], or in the cells overexpressing Sup35 PrD containing region fused to red fluorescent protein, RFP (Sup35NM-RFP), even though TTR does not contain a QN-rich domain, and M-TTR-GFP aggregation does not promote *de novo* formation of [*PSI*$^+$]. The M-TTR-GFP and Sup35NM-RFP aggregates colocalized the yeast cells, and Sup35 was co-immunoprecipitated with M-TTR-GFP aggregates. Interestingly, another yeast prion, formed by Rnq1 protein, [*PIN*$^+$] did not promote aggregation of M-TTR-GFP. One possibility that remains to be addressed is the nature of the Sup35-TTR coaggregates. Sup35 is known to undergo phase separation and form hydrogel-like assemblies at the acidic pH in yeast (Franzmann et al., 2018), thus it is possible that aggregates detected by Verma et al. could represent this kind of assemblies, although one counterargument against this is that prion state of Sup35 interferes with the incorporation of Sup35 into such assemblies rather than promotes them.

9. Yeast assay for amyloid nucleation by mammalian proteins

A new assay for the initial amyloid nucleation by mammalian proteins in yeast has been developed using *de novo* prion induction by overproduced constructs containing PrD of the yeast prion protein Sup35. As described above (see Section 2 and Fig. 3A), a transient overproduction of Sup35 or its PrD induces *de novo* formation of the [*PSI*$^+$] prion efficiently only in yeast cells containing other aggregated proteins, for example, the prion form of the Rnq1 protein, [*PIN*$^+$]. However, the addition of an expanded polyQ stretch to the Sup35 PrD-containing region enabled it to induce [*PSI*$^+$] after the transient overproduction in the strain lacking known pre-existing prions, [*psi*$^-$ *pin*$^-$]) (Goehler et al., 2010). As Sup35 PrD is also QN-rich, and expanded polyQ constructs are shown to promote [*PSI*$^+$] nucleation by excess Sup35 *in trans,* this result could in principle be interpreted as a lengthening or “duplication” of the Sup35 PrD region, that increases its prion-inducing abilities. Indeed, the construct combining the Sup35N (PrD) and Rnq1 sequences in a tandem was later shown to induce *de novo* [*PSI*$^+$] formation very efficiently (Newby et al., 2017). To check if an attachment of the amyloidogenic protein of an entirely different sequence composition, not capable of nucleating the Sup35 aggregation *in trans* would enhance *de novo* prion nucleation by Sup35 PrD, a series of constructs has been prepared (Chandramowlishwaran et al., 2018), in which either the Sup35N (PrD) or the Sup35NM (PrD with linker) coding region was fused in frame to the sequence, coding for one of the following amyloidogenic domains of mammalian origin: mouse PrP (the region between aa positions 90 and 230, known to be sufficient for the prion transmission as described in Section 6 above); human Aβ42; the aggregating core of human αSyn encompassing aa positions 61–95 (Irvine et al., 2008; Rivers et al., 2008), and the aggregation-prone region (aa positions 8–37) of human IAPP, or amylin (Louros et al., 2015; Westermark et al., 1990), a peptide hormone forming amyloid aggregates that are associated with type II diabetes (Fig. 10A). The chimeras were expressed from regulated promoters (copper-inducible P_{CUP1} or in some cases, galactose-inducible P_{GAL}) that allowed for the modulation of expression levels. Each of the chimeric constructs was able to nucleate [*PSI*$^+$] formation when transiently overproduced in the [*psi*$^-$ *pin*$^-$] yeast strain, lacking pre-existing prion (Chandramowlishwaran et al., 2018). The strongest effect was observed for Sup35N-Aβ42 construct, that was capable of nucleating

[*PSI*$^{+}$] in up to 12% of the cells at high levels of expression. The formation of detergent-resistant aggregates by chimeric proteins and the immobilization of full-length Sup35 into an aggregated state have also been confirmed by biochemical approaches. The insertion of the M region between the Sup35N and the mammalian sequences generally decreased [*PSI*$^{+}$] nucleation, however, all mammalian proteins mentioned above (with the exception of PrP) were still capable of nucleating [*PSI*$^{+}$] when fused to Sup35NM.

Notably, sequence alterations in PrP and Aβ that are known to antagonize prion propagation or amyloid formation also decreased the ability of respective constructs to nucleate the [*PSI*$^{+}$] prion in yeast, while the sequence alterations associated with a heritable form of the disease promoted [*PSI*$^{+}$] nucleation (Chandramowlishwaran et al., 2018). For example, a deletion of the region between aa positions 90 and 120 (which is known to be required for susceptibility to TSE, as described above) in PrP, or mutations disrupting intermolecular interactions involved in amyloid formation by Aβ (Hilbich, Kisters-Woike, Reed, Masters, & Beyreuther, 1992; Morimoto et al., 2004; Williams et al., 2004), such as triple substitution F19S, F20S, I31P also knocked out [*PSI*$^{+}$] nucleation by respective constructs in yeast (Chandramowlishwaran et al., 2018). Substitution K28E disrupting the proposed “salt bridge” in the Aβ structure (Reddy, Straub, & Thirumalai, 2009), and substitution Q167R in PrP, known to inhibit prion propagation in mice (Perrier et al., 2002), also decreased [*PSI*$^{+}$] nucleation in yeast (Chandramowlishwaran et al., 2018). The Aβ40 peptide, that is less amyloidogenic and less toxic in humans, as compared to Aβ42 (see above, Section 5.1), was also less efficient in nucleating [*PSI*$^{+}$] in yeast when fused to Sup35N, and was not capable of nucleating [*PSI*$^{+}$] when fused to Sup35NM. In contrast, the substitution P101L in mouse PrP (Manson et al., 1999), corresponding to P102L that is associated with heritable TSE in humans (Young et al., 1995), and the substitution D23N in human Aβ42, corresponding to so-called Iowa mutation, associated with the heritable form of AD (Grabowski, Cho, Vonsattel, Rebeck, & Greenberg, 2001; Van Nostrand, Melchor, Cho, Greenberg, & Rebeck, 2001) both increased [*PSI*$^{+}$] nucleation in yeast (Chandramowlishwaran et al., 2018). C-terminal truncations of mouse PrP also increased [*PSI*$^{+}$] nucleation in yeast; this agrees with the observations that similar truncations are associated with a heritable disease showing TSE symptoms in humans, even though infectivity of such truncated proteins has not been proven (Capellari et al., 2018; Kitamoto, Iizuka, & Tateishi, 1993; Lorenz, Windl, & Kretzschmar, 2002). Overall, these parallels between the yeast and mammalian models (summarized in Table 4) indicate that amyloidogenic properties of a mammalian protein drive prion nucleation by chimeric constructs in yeast, and confirm the relevance of yeast data to mammalian and human disease.

The exact molecular mechanism of prion nucleation by chimeric constructs remains under investigation. The most likely scenario is that mammalian proteins/domains aggregate in yeast thus bringing together the Sup35 PrD regions, attached to them, and therefore promoting the conversion of these regions into a cross-β nucleus (Chandramowlishwaran et al., 2018), as shown in Fig. 10B. The complete Sup35 protein, present in the cell is immobilized into such a nucleus and converted into a prion form, thus allowing a phenotypic detection. It is not clear whether the physical proximity of PrDs is sufficient for the initiation of amyloid conversion, or the attached cross-β assemblies formed by mammalian domains play an active role in the process. At least, an attachment of proteins forming non-amyloid

globular multimolecular assemblies to Sup35 PrD does not nucleate [*PSI*$^{+}$] formation at the level comparable to mammalian amyloidogenic domains (Chandramowlishwaran et al., 2018). Further studies are needed to determine if some proteins forming more complex, non-globular although still non-amyloid assemblies, such as liquid droplets, hydrogels, hydrophobic protein agglomerates or cytoskeletal fibrils, would have an effect on prion nucleation. However, independently of the outcome of these studies, it is obvious that the yeast nucleation assay could be applied to studying the effects of sequence alterations or chemicals on amyloid nucleation by known amyloidogenic proteins, as well as for identifying new candidate proteins with amyloid properties in various organisms.

The unique property of this assay is that it specifically targets the initial conversion from the non-amyloid into an amyloid form (a triggering step in amyloid diseases) and is capable of detecting amyloid abilities even for a protein that is not capable of propagating an amyloid state ion its own in the yeast cell. Our preliminary experiments using this approach have uncovered new mutations in Aβ that influence its amyloidogenic properties (O. Malikova, A. Rubel, and Y. Chernoff, unpublished data), detected new chemicals influencing amyloid nucleation by Aβ (P. Chandramowlishwaran, Z. Deckner, R. Mezencev and Y. Chernoff, unpublished data), and identified new proteins with amyloidogenic properties (confirmed by other methods) in a human proteome (A. Zelinsky, N. Romanova, D. Kachkin, A. Rubel and Y. Chernoff, unpublished data).

10. Conclusions and future directions

Yeast *S. cerevisiae* is a powerful model eukaryotic cell for studying the fundamental cellular processes and protein functions that are also associated with complex multicellular eukaryotes such as humans. The basic mechanisms and pathways leading to such manifestations of neurodegenerative diseases as transcriptional dysfunction, defect in trafficking, defect in clearance pathways such as proteasome or autophagy, mitochondrial dysfunction, transcriptional dysregulation *etc.,* are highly conserved between yeast and human species. Major features of protein misfolding and its consequences in yeast and mammalian cells exhibit a lot of similarities, and yeast can be easily manipulated genetically in order to investigate the role of prions and heritable amyloids associated with mammalian and human diseases. Due to huge cell numbers, simple cultivation techniques and availability of easily detectable phenotypes, level of resolution provided by yeast assays is unthinkable even for mammalian cell cultures, not mentioning animal models. As described above, yeast is specifically pliable for studying protein-based inheritance controlled by endogenous yeast prions that could be applied to characterizing mammalian proteins in specifically engineered constructs. Data reviewed above clearly demonstrate that yeast models have already made a huge contribution to understanding the molecular and cellular processes associated with major aggregation-related disorders. A number of important results obtained in yeast have been confirmed by further studies in animal models and/or human cells, and yeast assays have been successfully employed for identification of the anti-aggregation agents, some of which show a therapeutic promise.

The major issue usually raised in connection with applying yeast models to studying mechanisms of mammalian and human diseases is to which extent the behavior of

heterologous proteins in yeast cells recapitulates behavior of these proteins in their natural environments. This is a valid concern, however, it could be (and is being) addressed both experimentally, by rechecking the promising leads coming from yeast studies in animal models and human cells, and logically, based on already acquired knowledge about particular proteins and diseases. In application to amyloid and prion diseases, the pathway to disease can be divided into the following steps.

1. Initial nucleation of amyloid formation. In many cases, it can be reproduced by an amyloidogenic protein even *in vitro,* and therefore is primarily controlled by a protein itself. Thus, yeast cells provide an adequate model for studying the mechanisms underlying this crucial step, as well as for identifying conditions and agents, that influence initial amyloid nucleation and may uncover new leads for prophylactic recommendations or therapeutic interventions. Notably, availability of simple phenotypic assays and typically eukaryotic cellular environment make yeast models more useful for this kind of research even in comparison to *in vitro* biochemical studies. Up to date, this potential of yeast models for studying the initial amyloid nucleation has not yet been realized in its full capacity, although recent developments (for example, see Section 9 above) indicate that we may hope for significant breakthrough in these studies in near future.

2. Amyloid propagation and spread. Studying of endogenous yeast prions made seminal contributions to understanding these phenomena by uncovering the role of chaperone machinery in prion propagation, while extension of this work to heterologous mammalian proteins has already been fruitful and will likely continue to be so in future. However, some differences between the yeast and mammalian/human protein homeostasis machineries have already been noticed, and alteration of protein localization in yeast systems (*e.g.,* intracellular versus extracellular) may cause additional discrepancies. Here, validation of yeast data in authentic environments is certainly critical, although yeast simplicity still provides huge advantages for initial screens.

3. Mechanisms of amyloid toxicity. These mechanisms obviously vary with a particular disease and a particular protein, and applicability of yeast systems to understanding this step depends on the extent to which the mammal-specific parameters of pathology could be recapitulated in yeast cells. Some amyloidogenic proteins such as huntingtin-based constructs are toxic to yeast cells, and an argument can be made that at least some features of this toxic effect may recapitulate cytotoxicity observed in human disease. On the other hand, some aggregated mammalian proteins such as PrP appear not to be toxic to yeast cells, while other proteins such Aβ may cause toxicity by mechanisms that are different from those operating in humans. Thus, applicability of yeast model for studying specific processes leading to pathological manifestations could be limited, depending on a disease.

Overall, closer to the triggering step of the amyloid disease we are, more valuable are the advantages of a yeast-based assay, and more relevant are its outcomes for understanding the

processes occurring in mammalian and human organisms. This should be noted, however, that understanding the early steps of protein misfolding and spread represents the major challenge in counteracting protein assembly disorders, and targeting of this step would provide the most radical solution for the development of anti-amyloid therapies.

In addition to providing a model for studying amyloid diseases, that is primarily emphasized in the given review, yeast assays could also be applied to identifying new mammalian and human amyloids or other aggregated proteins that could be involved in regulatory processes. This direction of research is only at the beginning steps, and future studies promise interesting new developments in this area.

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Abbreviations

25Q	huntingtin polyQ tract containing 25 glutamine residues
25QP	huntingtin polyQ tract containing 25 glutamine residues and followed by the P-rich region
103Q	huntingtin polyQ tract containing 103 glutamine residues
103QP	huntingtin polyQ tract containing 103 glutamine residues and followed by the P-rich region
αSyn	α-synuclein
aa	amino acid residue
AAA+	the “ATPases associated with diverse cellular activities” protein superfamily
AD	Alzheimer’s disease
ALS	amyotrophic lateral sclerosis
APP	amyloid precursor protein
ATTR	transthyretin amyloidosis
Apβ	amyloid beta
BSE	bovine spongiform encephalopathy
CFP	cyan fluorescent protein
eRF1	eukaryotic release factor 1 (termed Sup45 in yeast)
eRF3	eukaryotic release factor 3 (termed Sup35 in yeast)

fALS	familial amyotrophic lateral sclerosis
FRET	fluorescence resonance energy transfer
FTD	frontotemporal dementia
FTDP-17	frontotemporal dementia with parkinsonism associated with chromosome 17
GFP	green fluorescent protein
GPI	glycophosphatidylinositol
HD	Huntington's disease
hnRNP	heterologous nuclear ribonucleoprotein
Hsp	heat shock protein
Htt	huntingtin protein
Htt-GFP	exon 1 of Htt, fused to GFP
IAPP	islet amyloid polypeptide, also termed amylin
IMS	intermembrane space (mitochondrial)
IPOD	insoluble protein deposit
kD	kilodalton
KMO	kynurenine 3-monooxygenase
LCD	low complexity domain
MAPT	microtubule-associated protein tau
MRF	the region including middle (M) and release factor (RF, or C) domains of Sup35 protein
MSP	multisystem proteinopathy
NAB	N-aryl benzimidazole
NFT	neurofibrillary tangle
NQ stretch	asparagine/glutamine-rich stretch in the yeast Sup35 protein
NR	region of oligopeptide repeats in the yeast Sup35 protein
NLS	nuclear localization signal
PD	Parkinson's disease
PHF	paired helical filament

PolyQ	polyglutamine
PolyQP	construct including the polyglutamine tract of huntingtin protein, followed by the P-rich region
PrD	prion domain
PrLD	prion domain like domain
PrP	mammalian prion protein
PrP^{Sc}	“scrapie” or prion isoform of mammalian prion protein PrP
PrP^{C}	cellular or non-prion isoform of mammalian prion protein PrP
RF	release factor C-proximal region of the yeast protein Sup35
RFP	red fluorescent protein
RRM	RNA recognition motif
SDS	sodium dodecyl sulfate
SDD-AGE	semi-denaturing detergent agarose gel electrophoresis
SG	stress granule
SPB	spindle body
TAR	trans-activation response element
ThT	thioflavin-T
TSE	transmissible spongiform encephalopathy
TTR	transthyretin protein
YFP	yellow fluorescent protein

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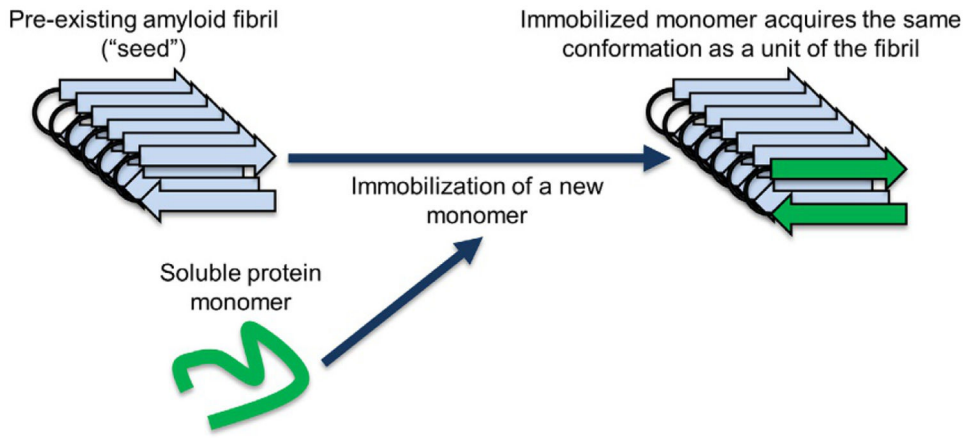

Fig. 1.

Templated nucleated polymerization of amyloids and prions. The example of parallel in-register cross-β amyloid structure (β-arch) is shown. Boxes with arrowheads correspond to β-strands. The folded intermolecular β-sheet exists only within a polymer. A newly immobilized monomer acquires exact same conformation as a pre-existing unit of the amyloid fibril due to formation of hydrogen bonds between identical amino acid (aa) residues.

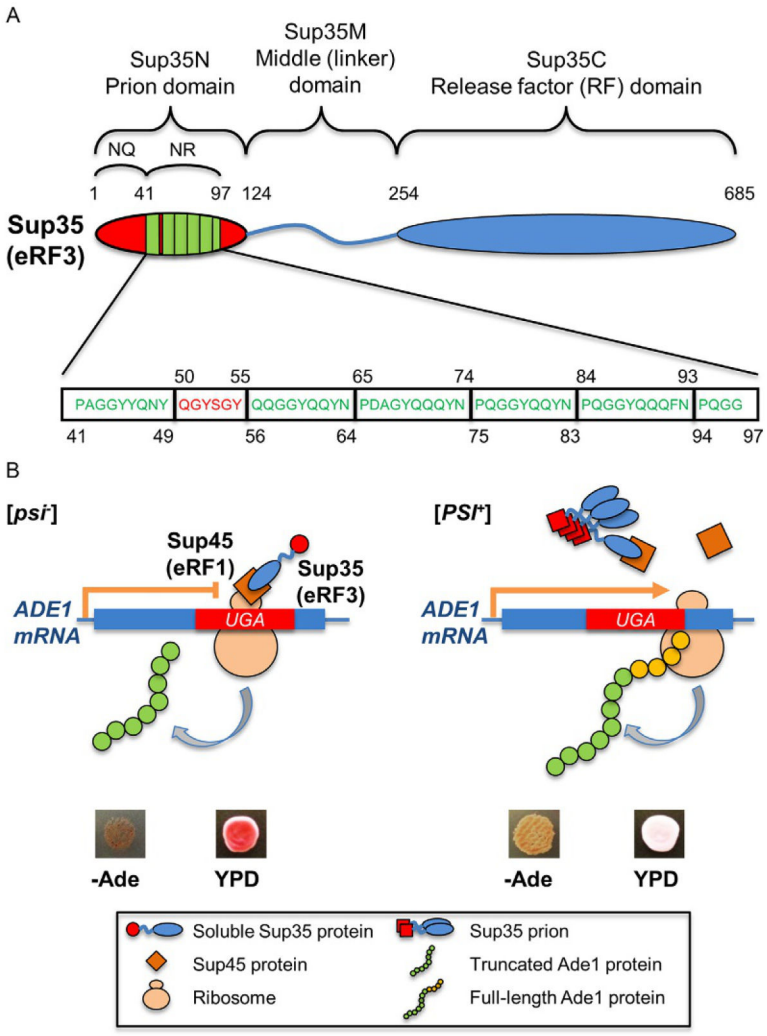

Fig. 2.

The Sup35/[*PSI*$^+$] system in yeast. (A) Structural and functional organization of the yeast Sup35 protein. NQ—asparagine- and glutamine-rich stretch, NR—region of oligopeptide repeats. Repeats are indicated by green boxes. Sequences of oligopeptide repeats are shown by green characters, the piece of the sequence located between the first and second repeats—by red characters. Numbers correspond to aa positions. (B) The phenotypic detection assay for the [*PSI*$^+$] prion. On the left—soluble Sup35 (eRF3), together with Sup45 (eRF1), is functioning as a part of translation termination complex in the [*psi*$^-$] strain bearing the premature stop codon in the *ADE1* gene (UGA nonsense-allele *ade1-14*). Termination on this premature stop codon results in the formation of truncated Ade1 protein, leading to the inability to grow on the medium lacking adenine (−Ade) and red color (due to accumulation of the red pigment, which is a polymerized intermediate of the adenine biosynthetic pathway) on the complete organic (YPD) medium. On the right—aggregation of Sup35 in the prion-containing ([*PSI*$^+$]) cells, accompanied by sequestration of Sup45, decreases the ability of the termination complex to access translating ribosomes, and results in the impairment of termination, leading to the readthrough (nonsense-suppression) of premature

UGA codon and synthesis of full-length Ade1 protein, that confers growth on −Ade medium and prevents accumulation of the red pigment on YPD medium. Designations of the soluble and aggregated (prion) forms of Sup35, as well as designations of Sup45, ribosome and newly synthesized Ade1 polypeptide are indicated.

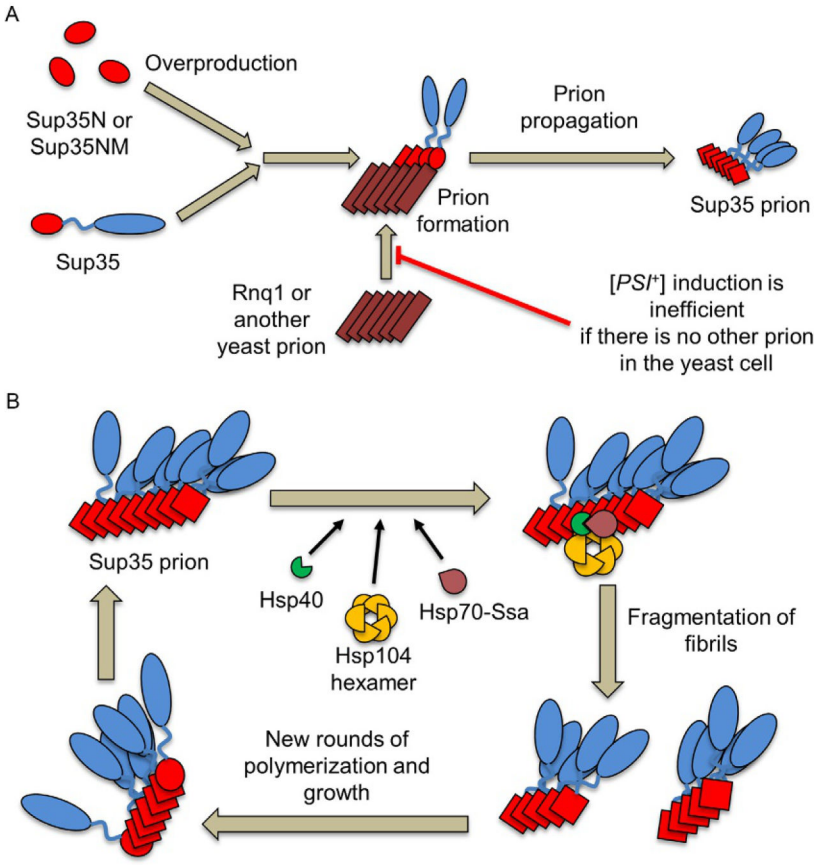

Fig. 3.

[*PSI*$^{+}$] formation and propagation in yeast, and roles of other proteins. (A) Induction of [*PSI*$^{+}$] formation by overproduction of constructs bearing Sup35 PrD (Sup35N or NM), in the presence of another yeast prion (such as Rnq1 prion, [*PIN*$^{+}$]) acting as a heterologous nucleation center. (B) Chaperone role in [*PSI*$^{+}$] propagation: fragmentation of amyloid fibrils, generating new oligomeric "seeds" for new rounds of polymerization is achieved by the chaperone machinery composed of the Hsp104, Hsp70-Ssa and Hsp40 proteins. Designations of the prion and non-prion isoforms are the same as in Fig. 2. See more detailed comments in the text.

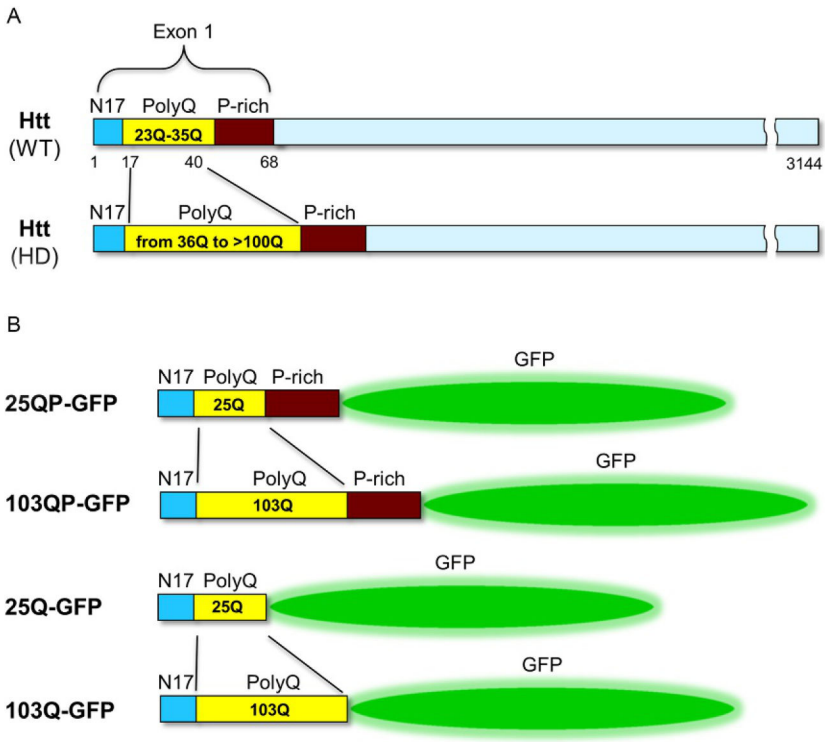

Fig. 4.

Huntingtin protein (Htt) and its derivatives used to model Huntington's disease (HD) in yeast. (A) Human full-length wild-type (WT) and Huntington disease-associated (HD) variants of the Htt protein. Aa numbering is shown for the variant with 23 glutamines (Qs) in the polyglutamine (PolyQ) tract. N17—amino terminal region of 17 aa; P-rich—proline-rich region. (B) Yeast constructs for studying polyQ aggregation and toxicity. For the polyQ-expanded version, only a construct with the longest polyQ stretch (designated as 103Q, see explanation in the text) is shown as an example. Yeast constructs with shorter polyQ expansions are also used as described in the text. GFP—green fluorescent protein (the most frequently used fluorophore, although other fluorophores are also occasionally employed). This should be noted that majority of the yeast Htt-derived polyQ constructs also contain the FLAG epitope attached at the N-terminus (not shown in figure).

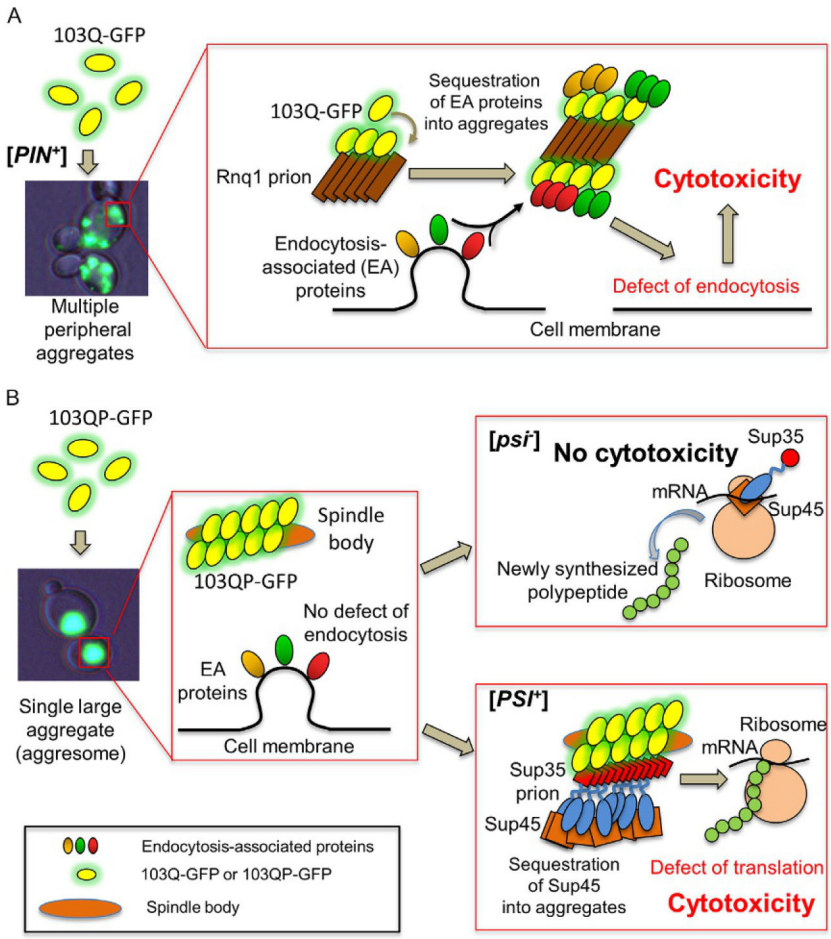

Fig. 5.

Role of endogenous yeast prions in polyQ aggregation and cytotoxicity. (A) Rnq1 aggregates nucleate formation of multiple peripheral 103Q-GFP aggregates in the [*PIN*$^+$] strain, containing Rnq1 protein in a prion form. Sequestration of the endocytosis-associated (EA) proteins by 103Q-GFP aggregates leads to the defect of endocytosis, resulting in cytotoxicity. Other proteins, sequestered by polyglutamine aggregates and possibly contributing to cytotoxicity are discussed in the text. (B) 103QP-GFP protein, containing the P-rich region (see Fig. 4), is assembled into a cytoprotective aggregate deposit (aggresome), colocalized with a spindle body. This prevents sequestration of EA proteins and makes constructs non-toxic to [*psi*$^-$] cells, containing Sup35 protein in a non-prion form. However, in the [*PSI*$^+$] cells, which contain the prion form of Sup35 protein, 103QP-GFP polymers sequester aggregated Sup35 (and through it, another translation termination factor, Sup45), leading to the defect of translation, that results in cytotoxicity. Designations unique for this figure are shown in the bottom left corner;other designations are the same as on Fig. 2.

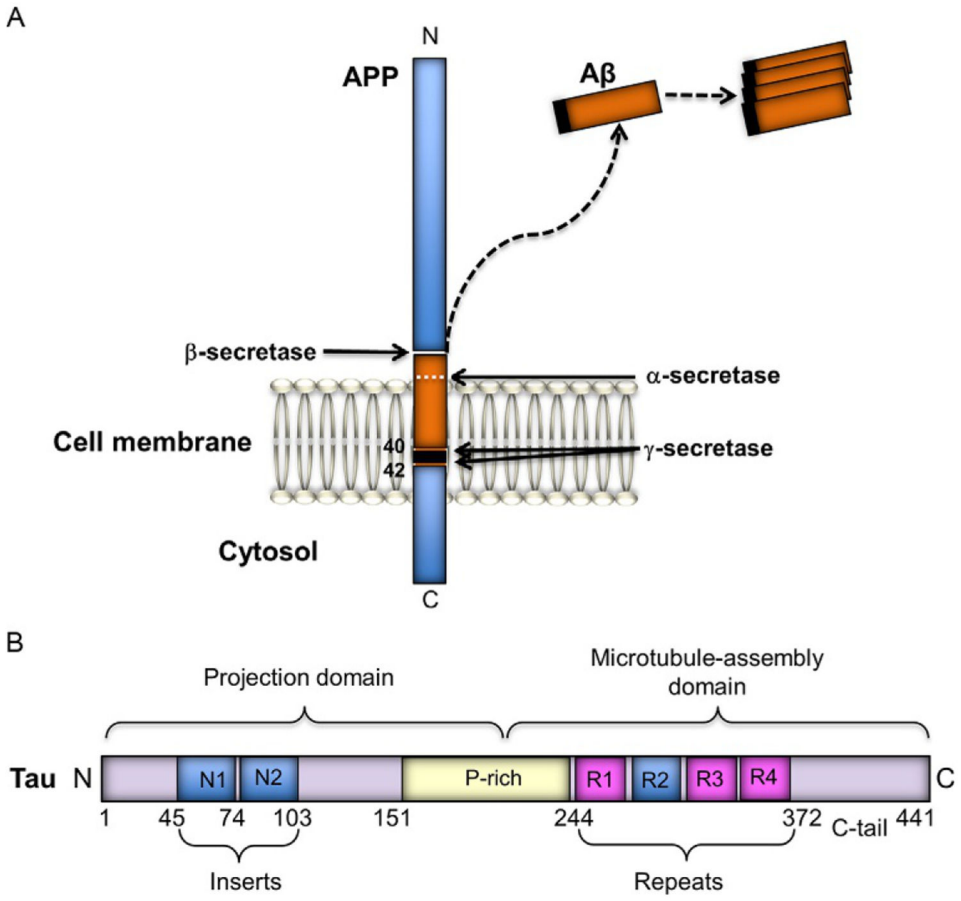

Fig. 6.

Aβ and tau proteins. (A) Generation of Aβ by proteolytic processing of the amyloid precursor protein (APP). APP processing is catalyzed by the membrane-associated secretase complex. Cleavage by β-secretase and subsequently, by γ-secretase produces Aβ peptides, while cleavage by α-secretase prevents Aβ formation. Depending of the position of β-secretase cleavage site, Aβ peptides of various lengths are produced. Two major sites, leading to the formation of 40 aa (Aβ40) and 42 aa (Aβ42) peptides are indicated. An example of Aβ42 production as well as its subsequent polymerization are shown. (B) Structural and functional organization of the longest isoform of human tau protein of 441 aa in length (tau441 or tau 2N4R) presented in the neurons of central nervous system is shown. Alternative splicing may eliminate some or all of the regions shown in blue rectangles, resulting in the generation of total of six tau isoforms, denoted by either their total number of amino acids or the number of N-terminal exons (N) and microtubule-associated repeats (R). Exons absent in some of the shorter isoforms but present in the longest isoform (N1 and N2) are termed “Inserts.” The N-terminal part tau is referred to as the “Projection domain” since it projects away from the microtubule surface and can interact with membrane-associated structures or motor proteins. Microtubule-assembly domain containing repeat sequences (R1-R4 in the longest isoform), and adjacent proline-rich (P-rich) region are also indicated. These regions of tau regulate the rate of microtubule polymerization. Repeat sequences are also involved in the formation of tau amyloid fibrils.

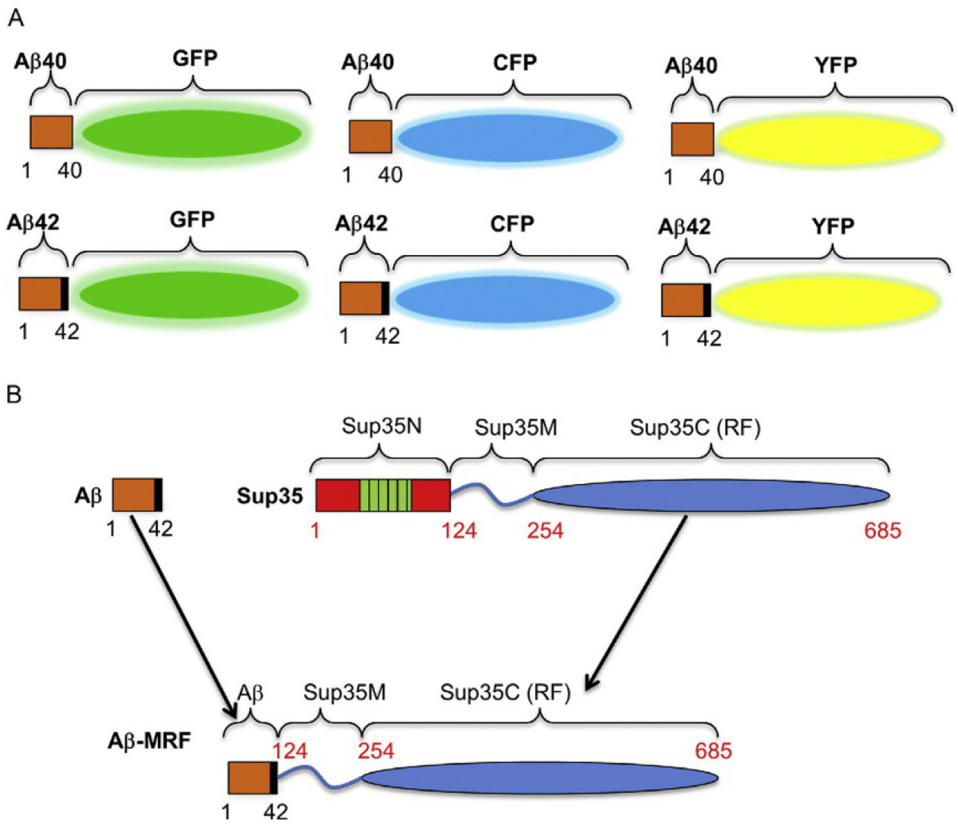

Fig. 7.

Examples of yeast model systems for the detection of Aβ aggregation. (A) Fusion of Aβ to a fluorophore: C-terminal fusions of Aβ40 and Aβ42 to green (GFP), yellow (YFP) or cyan (CFP) fluorescent proteins are shown. (B) Chimeric construct for the phenotypic detection of Aβ aggregation, using the yeast Sup35 protein (translation termination factor) as a reporter in a termination readthrough (nonsense-suppression) assay described on Fig. 2. In this construct, Aβ42 is substituted for the PrD region of Sup35 (Sup35N). Resulting chimeric protein, retaining the middle (Sup35M) and the C-proximal release factor (RF, Sup35) domains of Sup35 is termed Aβ42-MRF. Designations of the Sup35 domains are the same as on Fig. 2; Aβ designations are the same as on Fig. 6. Numbers indicate amino acid positions in Aβ (black font, located under the drawing in a chimeric construct) and Sup35 (red font, located above the drawing in a chimeric construct).

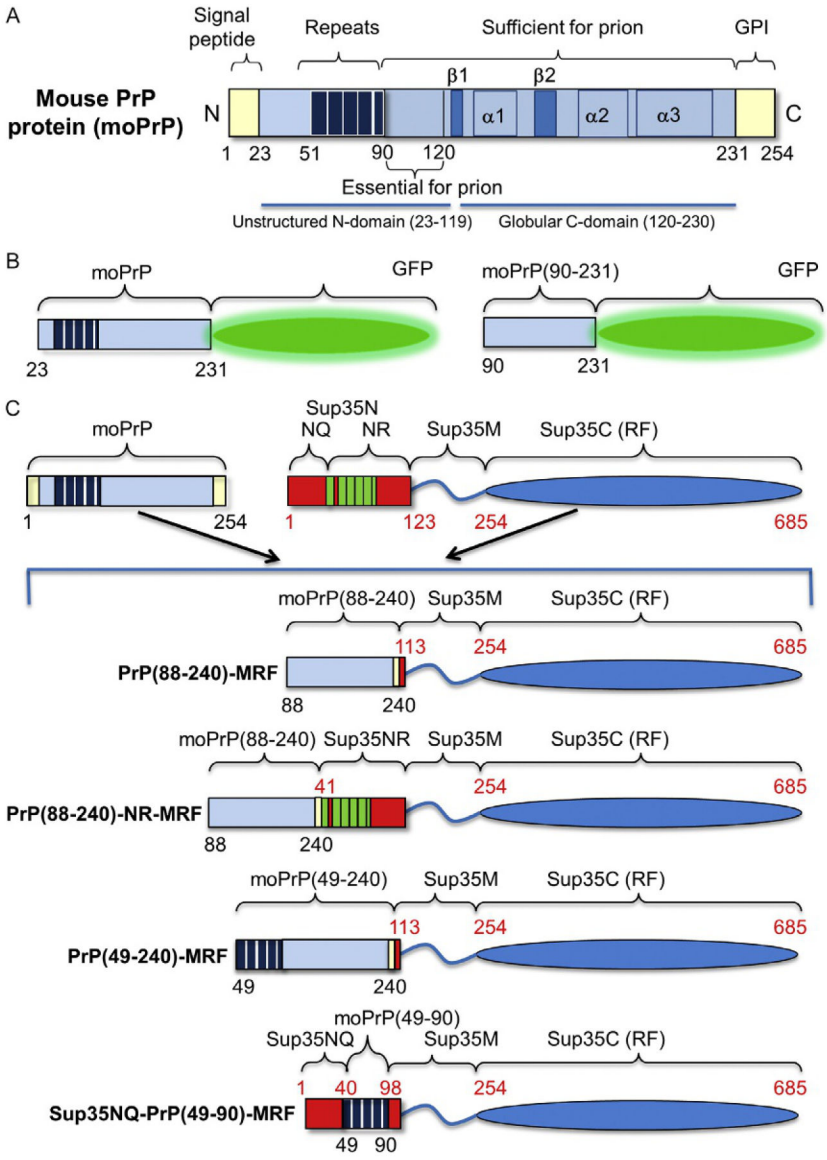

Fig. 8.

Mouse prion protein (PrP) and its derivatives used in yeast studies. (A) Structural and functional organization of mouse PrP(moPrP). Signal peptide (first 22 aa), which is cleaved during processing, as well as the region including G-rich octapeptide repeats (indicated by navy blue boxes), and a GPI anchoring signal (GPI) are indicated. The processed form of moPrP (23–230) includes the N-proximal unstructured domain and C-terminal globular domain as shown. The globular domain contains three α-helices (α1–3), and two β-strands (β1–2) as indicated. Glycosylation sites at positions 180 and 196 (not recognized in yeast), and a disulfide bridge are not shown. Regions that are essential and sufficient for prion propagation in mammals are indicated. This should be noted that the last (incomplete) oligopeptide repeat overlaps with the region 90–119 that is crucial for prion propagation. (B) C-terminal fusions of mature full-length (23—231) and N-terminal truncated (90–231) moPrP with GFP. (C) Construction of chimeric moPrP-Sup35 proteins. Designations of the

Sup35 regions are the same as on Fig. 2 (with the Sup35N domain shown as a rectangle, as it can aggregate when included in a chimeric construct). Numbers indicate amino acid positions in moPrP (numbers located under the drawing in a chimeric construct, shown in black font) and Sup35 (numbers located above the drawing in a chimeric construct, shown in red font). Chimeras beginning from the PrP-derived sequence contained four N-terminal amino acids of Sup35N remaining in the chimeric construct (not shown in figure).

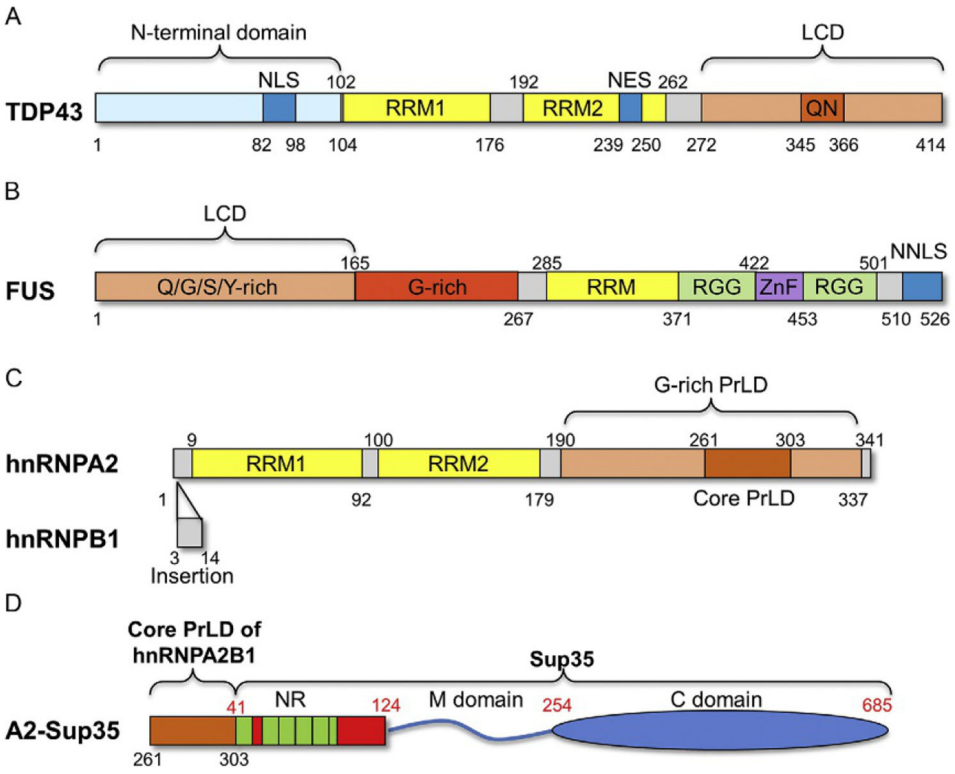

Fig. 9.

Proteins associated with amyotrophic lateral sclerosis (ALS). (A) Structural and functional organization of TDP-43. NLS—nuclear localization signal;RRM1 and RRM2—RNA recognition motifs 1 and 2, respectively; NES—nuclear export signal; LCD—low complexity domain; QN—QN-rich domain. (B) Structural and functional organization of FUS. Q/G/S/Y-rich—the region rich in glutamine, glycine, serine, and tyrosine; G-rich—the region rich in glycine, RRM—RNA recognition motif, RGG—the motifs containing arginine/glycine/glycine repeats; ZnF—zinc finger domain; NNLS—non-conventional nuclear localization signal. (C) hnRNPA2B1 and yeast chimeric constructs based on this protein: PrLD –prion domain like domain; Core PrLD—core region of PrLD; RRM1 and RRM2—RNA recognition motifs 1 and 2, respectively. Insertion distinguishing hnRNPB1 from hnRNPA2 is shown. Sup35 designations are the same as on Fig. 2 (with the Sup35N domain drawn as a rectangle, as it aggregates when included in a chimeric construct). Numbers indicate amino acid positions in ALS-associated proteins (black font, located under the drawing in a chimeric construct) and Sup35 (red font, located above the drawing in a chimeric construct).

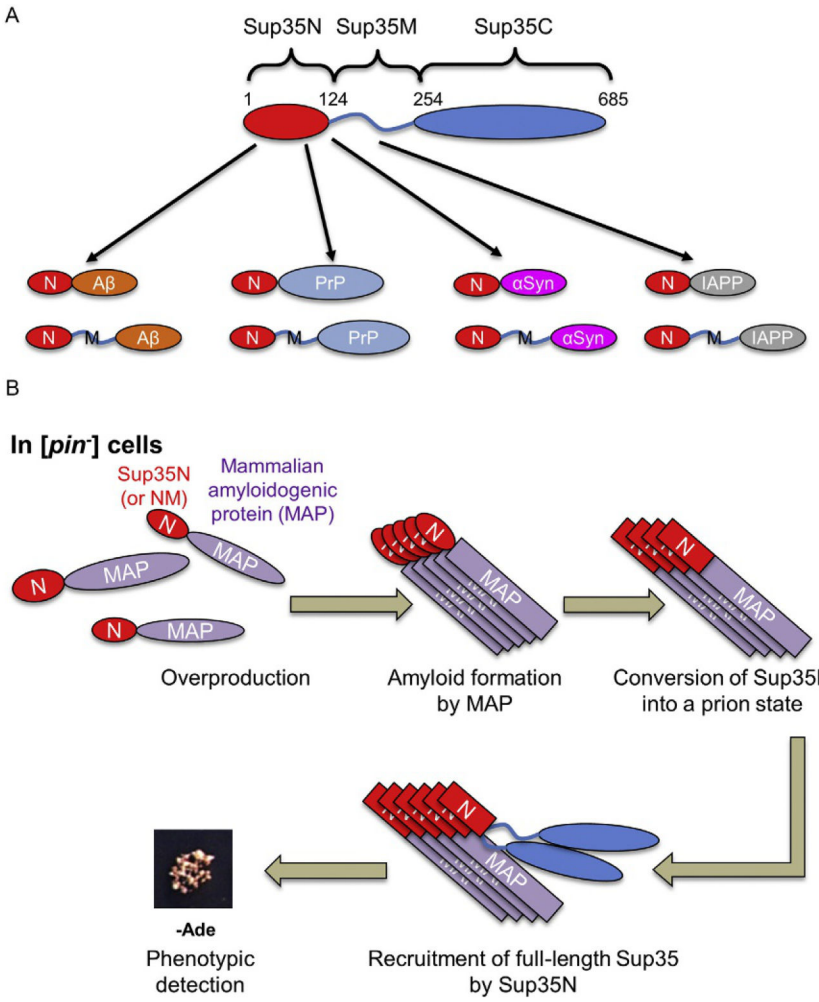

Fig. 10.

Prion nucleation by mammalian amyloidogenic proteins (MAPs) in yeast. (A) Chimeric prion domains constructed from the Sup35 PrD-containing region fused to Aβ, PrP, αSyn and IAPP, respectively (see text for the description of the regions of amyloidogenic proteins, used in these constructs). (B) Model of *de novo* prion nucleation by chimeric constructs in the [*pin*$^{-}$] yeast cells lacking any known pre-existing prions. As shown in Fig. 3, overexpression of Sup35N or Sup35NM alone does not lead to efficient nucleation of the [*PSI*$^{+}$] prion in the [*pin*$^{-}$] cells. Designations of the domains, prion and non-prion forms of Sup35 are the same as on Fig. 2. The region of oligonucleotide repeats (NR) is present, but not shown for the sake of simplicity. See text for detailed description.

Table 1

Examples of mammalian and human disease-associated misfolded proteins that have been studied in yeast.

Misfolded protein(s)	Localization	Disease	Type
Huntingtin (Htt)	Cytoplasmic or nuclear	Huntington's disease (HD)	Heritable
α-Synuclein (αSyn)	Cytoplasmic	Parkinson's disease (PD) and other synucleinopathies	Mostly sporadic, sometimes heritable
Amyloid β (Aβ)	Mostly extracellular	Alzheimer's disease (AD)	Primarily sporadic, rarely heritable
Tau	Cytoplasmic	AD, frontotemporal dementia (FTD), Pick's disease and other tauopathies	Sporadic or heritable
Prion protein (PrP)	Mostly extracellular	Transmissible spongiform encephalopathies (TSEs)	Sporadic, heritable and/or infectious
TDP-43	Cytoplasmic or nuclear	Amyotrophic lateral sclerosis (ALS), FTD	Sporadic or heritable
FUS	Cytoplasmic or nuclear	ALS	Sporadic or heritable
C9orf72	Cytoplasmic	ALS, FTD	Heritable
Cu,Zn-superoxide dismutase (Sod1)	Cytoplasmic, or mitochondrial IMS[a]	ALS	Sporadic or heritable
hnRNPA2B1, hnRNPA1	Nuclear or cytoplasmic	Multisystem proteinopathy (MPS)—type of ALS	Heritable
CREST	Nuclear	ALS	Heritable
VAPB, VCP, OPTN, SETX, profilin 1	Cytoplasmic or nuclear	ALS	Heritable
Ataxin 2 (ATXN2)	Nuclear or cytoplasmic	Spinocereberral ataxia 2 (SCA), ALS, FTD	Heritable
Transthyretin (TTR)	Extracellular or cytoplasmic	Transthyretin amyloidosis (ATTR), polyneuropathy	Mostly heritable
Amylin (IAPP)	Mostly extracellular	Type II diabetes	Sporadic

[a]Intramembrane space.

Table 2

Examples of yeast and fungal amyloid-based prions.

Species and protein	Cellular function	QN-rich PrD(s)	Prion designation
Saccharomyces cerevisiae			
Sup35 (eRF3)	Translation termination (release) factor	Yes	[*PSI*$^+$][a]
Ure2	Regulatory protein in nitrogen metabolism	Yes	[*URE3*][b]
Rnq1	Unknown	Yes	[*RNQ*$^+$] or [*PIN*$^+$][c]
Swi1	Chromatin remodeling factor	Yes	[*SWI*$^+$][d]
Cyc8	Transcriptional corepressor	Yes	[*OCT*$^+$][e]
Mot3	Transcriptional repressor	Yes	[*MOT3*$^+$][f]
Nup100	FG-nucleoporin	Yes	[*NUP100*$^+$][g]
Lsb2	Stress-inducible cytoskeletal protein	Yes	[*LSB*$^+$][h]
Mod5	tRNA isopentenyltransferase	No	[*MOD*$^+$][i]
Podospora anserina			
Het-s	Cytoplasmic incompatibility	No	[*Het*^{S}][j]

[a] Cox (1965) and Wickner (1994).

[b] Lacroute (1971) and Wickner (1994).

[c] The term [*PIN*$^+$], from "[*PSI*$^+$] inducibility" has initially been introduced to designate the specific prion factor that promoted *de novo* formation of [*PSI*$^+$] (Derkatch, Bradley, Zhou, Chernoff, & Liebman, 1997) and has later been identified as a prion form of Rnq1 protein (Derkatch, Bradley, Hong, & Liebman, 2001). Rnq1 prion was termed [*RNQ*$^+$] in an independent paper (Sondheimer, Lopez, Craig, & Lindquist, 2001). Other prions may also exhibit Pin$^+$ phenotype.

[d] Du, Park, Yu, Fan, and Li (2008).

[e] Patel, Gavin-Smyth, and Liebman (2009).

[f] Alberti, Halfmann, King, Kapila, and Lindquist (2009) and Holmes, Lancaster, Lindquist, and Halfmann (2013).

[g] Halfmann, Wright, Alberti, Lindquist, and Rexach (2012).

[h] Chernova, Kiktev, et al. (2017).

[i] Suzuki, Shimazu, and Tanaka (2012).

[j] Coustou, Deleu, Saupe, and Begueret (1997) and Maddelein, Dos Reis, Duvezin-Caubet, Coulary-Salin, and Saupe (2002).

Table 3

Comparison of yeast models for studying Aβ aggregation.

Yeast construct	Localization	Amyloid aggregation	Toxicity	Soluble to aggregate switch	Express detection of aggregation in yeast
Aμ-GFP (−YFP,−CFP)	Cytoplasmic	Instant	No	No	Cytological (by fluorescence)
GFP-Aμ	Cytoplasmic	Instant	Yes	No	Cytological (by lack of fluorescence)
MFα-Aμ42-GFP	Secretory pathway or periplasmic	?[a]	Yes	?	?[a]
ssAμ42-GFP	Secretory pathway or periplasmic	Instant?	Yes	?	?[b]
Aμ-MRF (Aμ-Sup35MC)	Cytoplasmic	Instant	No	No	Phenotypic (by readthrough)
Sup35N (NM)-Aμ[c]	Cytoplasmic	Inducible	No	Yes	Phenotypic (by readthrough)

[a]Authors detect accumulation of fluorescent protein, but it is not clear if it is in an amyloid form, or is simply accumulated within vesicular compartments (D'Angelo et al., 2013).

[b]Authors confirm formation of detergent-resistant aggregates by biochemical means, however, the fluorescence microscopy assay detects protein accumulation in the secretory pathway, rather than amyloid-type aggregation *per se* (Matlack et al., 2014).

[c]Data are from Chandramowlishwaran et al. (2018) as described in Section 9.

Table 4

De novo [*PSI*$^{+}$] nucleation by wild-type and altered derivatives of Aβ and PrP in yeast.[a]

Protein	Derivative	Effect *in vitro* or in mammals/humans	Effect in yeast when fused to Sup35 PrD
PrP	90–230	Susceptible to TSE	Prion nucleation
	23–230	Susceptible to TSE, prone to instability	Increased prion nucleation
	120–230	Not susceptible to TSE	No prion nucleation
	90–144	Heritable TSE-like disease[b]	Increased prion nucleation
	90–159	Heritable TSE-like disease[b]	Increased prion nucleation
	90–171	Not tested[c]	Increased prion nucleation
	90–230 P101L	Heritable TSE	Increased prion nucleation
	90–230 Q167R	Inhibition of PrP^{Sc} propagation	Decreased prion nucleation
Aβ	1–42	High aggregation propensity	Prion nucleation
	1–40	Low aggregation propensity	Low prion nucleation
	1–42 19S, F20S,I31P	No amyloid formation[d]	No prion nucleation
	1–42 D23N	Heritable AD	Increased prion nucleation
	1–42 K28E	Aβ structure impairment?	Decreased prion nucleation

[a] Yeast data are from Chandramowlishwaran et al. (2018). See text for mammalian and *in vitro* references.

[b] Effects of truncations in mammalian/human systems were studied within the context of a protein containing the full-length N-proximal region.

[c] While this particular truncation has not been studied in mammals, it has been reported (after publication of yeast data) that a truncation at the aa position 169 of human PrP is associated with a TSE-like disease (Capellari et al., 2018).

[d] Individual substitutions were also tested in yeast, with a strongest effect detected for I31P.