Mycoscience (2008) 49:221–228
DOI 10.1007/s10267-008-0413-9
© The Mycological Society of Japan and Springer 2008

REVIEW

Takao Ishikawa

Recent advances of research on the [*PSI*+] prion in *Saccharomyces cerevisiae*

Received: December 30, 2007 / Accepted: February 7, 2008

Abstract Prion diseases such as bovine spongiform encephalopathy or Creutzfeldt–Jakob disease have been extensively studied in recent years. Research in this field is being done in highly secured laboratories because of potential transmission of prions to humans. Emerging similarities between mammalian and yeast prions allow using yeast-based assays to examine the activity of anti-prion drugs. Besides the intensively studied clinical aspects of prion diseases, the evolutionary aspects of prion proteins present in the yeast *Saccharomyces cerevisiae* are also extensively investigated. One of the key feature of prions, the ability to be stable in two alternative conformations, seems to play an important role in the evolution of this fungi, although some authors point out the negative influence of these particles upon yeast physiology. In this review, the most intensively studied fields of the research carried out on [*PSI*+] prion in yeast are summarized.

Key words Drug screening · Prion · Translation termination · Yeast evolution

Introduction

The term "prion" was used for the first time to describe proteinaceous infectious particles causing transmissible spongiform encephalopathies and distinguish them from viruses (Prusiner 1982). Nowadays, it is widely known that prions can adopt at least two alternative and stable conformations. The prion protein in its normal cellular conformation may, for various reasons, alter the three-dimensional structure without any changes in the protein sequence. Proteins showing this feature have been identified in a number of mammalian species and designated as PrP (Prusiner 1994). However, the physiological role of prions is still the subject of intensive research. It is worth mentioning that the conformational changes are reversible, yet in case of mammalian prions only in vitro (Callahan et al. 2001). Reversion to the normal conformation of prion protein cannot take place under physiological conditions, and for this reason the prion diseases are always fatal. The research on prions entered a new phase with the discovery of prion-like factor [*PSI*+] in the yeast *Saccharomyces cerevisiae* (Wickner 1994; Sondheimer and Lindquist 2000). At this point it should be stressed that neither the structure nor the function of yeast prions can be linked with those of the mammalian prions. For a comprehensive review of fungal prions, see Benkemoun and Saupe (2006).

The properties of yeast prions are comparatively well known, and the cellular function of these proteins is often determined. It has also been noticed that the conformational changes of proteins frequently lead to a loss of function that can be observed as phenotypic changes in yeast cells. There are data suggesting the correlation between dysfunction caused by conformational changes and aggregation of protein. Upon cell division cytosol is, together with aggregated prion proteins, divided between two daughter cells. Prion proteins facilitate conformational changes of normal proteins in daughter cells after each division. Hence, prion particles are not diluted by cell divisions and are maintained over many rounds of this process. For this reason, the phenotype caused by prion is heritable.

Discovery of the [*PSI*+] prion

One of the most studied yeast prions is a determinant of the [*PSI*+] phenotype, characterized by readthrough of the stop codon, reported for the first time by Cox (1965) in work regarding suppressors of nonsense mutations. It became clear that the frequency of readthrough of stop codons is increased by the presence of cytoplasmic [*PSI*+] factor, although its nature remained unclear. Later it was shown that a nonsense-suppression phenotype similar to the [*PSI*+]

T. Ishikawa (✉)
Department of Molecular Biology, Institute of Biochemistry, Faculty of Biology, University of Warsaw, 1 Miecznikowa St., PL-02-096 Warsaw, Poland
Tel. +48-22-5543105; Fax +48-22-5543116
e-mail: takao@biol.uw.edu.pl

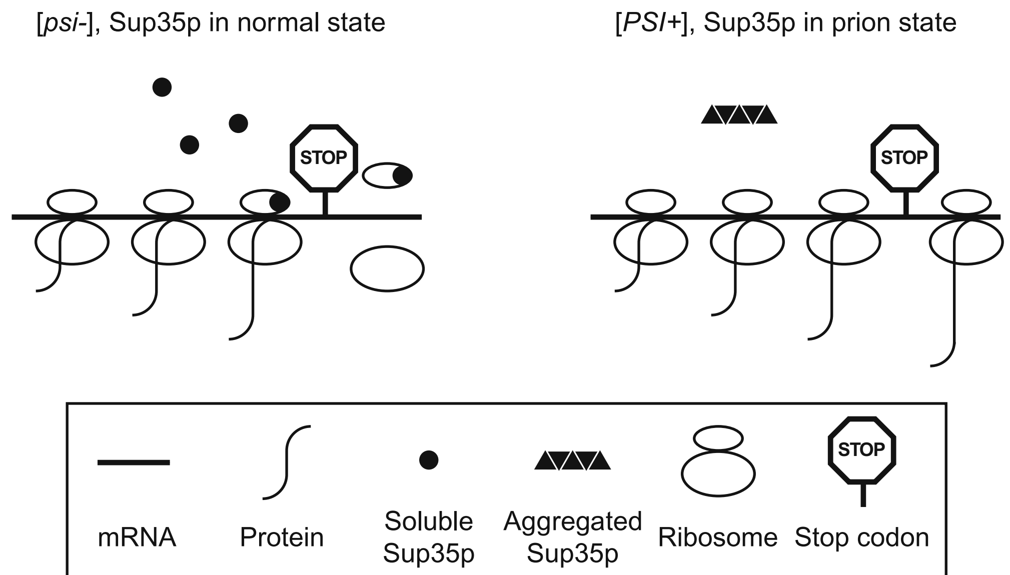

Fig. 1. Molecular basis of the [*PSI*+] phenotype. Symbols are identified in the *box* at the bottom of the figure

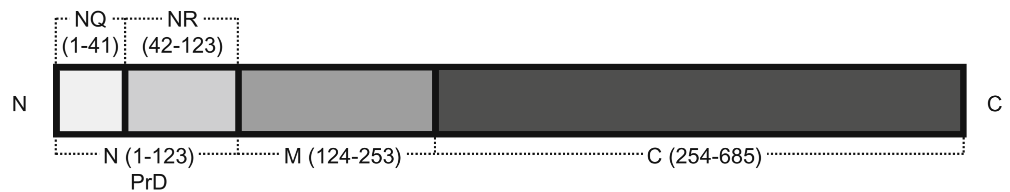

Fig. 2. Domain organization of the Sup35 protein

phenotype may be caused by mutations in the *SUP35* gene (Serio and Lindquist 1999, 2000). However, a segregation ratio of the phenotype caused by mutations was 2:2, while [*PSI*+] factor was observed in all daughter cells. Further, it was shown that the transfer of cytoplasm from the [*PSI*+] cells to the wild-type cells designated as [*psi*–] induces the phenotypic change (Cox et al. 1988). It could be explained by the existence of the cytoplasmic DNA, but further investigations indicate that change of phenotype from [*psi*–] to [*PSI*+] is independent of 2μ plasmid, mitochondrial DNA, or 20S RNA (Young and Cox 1972; Tuite et al. 1982; Cox et al. 1988). On the other hand, mild denaturating detergents were able to decrease the rate of readthrough of the stop codon. It leads to the conclusion that [*PSI*+] factor is a cytoplasmic proteinaceous agent present in multicopy form. As a consequence of these facts, the hypothesis was proposed that [*PSI*+] factor is in fact the Sup35 protein in the prion state (Wickner 1994). The Sup35p is an essential member of the translation termination complex (Fig. 1). The foregoing hypothesis, together with other results, seemed to explain observations made by Cox (1965) well.

According to this hypothesis, the Sup35 protein in [*psi*–] cells remains functional because of its native conformation (Sup35p^{psi-}), while in [*PSI*+] cells the same protein with altered structure is dominant (Sup35p^{PSI+}). Additionally, it was supposed that Sup35p^{PSI+} can induce the conformational changes of Sup35p^{psi-} into Sup35p^{PSI+} (Wickner 1994). This assumption was supported by de novo appearance of the [*PSI*+] phenotype in cells with the overexpression of the Sup35p (Chernoff et al. 1993). These data also suggest that spontaneous conversion of Sup35p structure is, not surprisingly, more likely to occur under its high concentration (Wickner 1994). For a review of the translational bypass of stop codons in yeast, see von der Haar and Tuite (2007).

The other important discovery was one of the mutations in the *SUP35* gene, designated as *PNM2* (an abbreviation of *Psi no more*) (Young and Cox 1971). This mutation is only a change of the 58th residue of the Sup35p from glycine to aspartic acid, which eliminates the [*PSI*+] phenotype (Doel et al. 1994). Today, the region of the protein containing the 58th residue is identified as the N domain, which is responsible for the appearance of two alternative and stable three-dimensional conformations of the Sup35 protein.

Structural organization of Sup35p

The Sup35 protein is built up from 685 amino acid residues, which can be divided into three domains: N, M, and C (Kushnirov et al. 1988; Ter-Avanesyan et al. 1993). The core part of the protein is made from the C domain (residues 254–685), which contains the region responsible for the translation termination. The rest of the Sup35p contains domains N (residues 1–123) and M (residues 124–253). The former causes the whole protein to act as a prion, and therefore the N domain is also frequently described as a prion-forming domain (PrD) (Ter-Avanesyan et al. 1994). The M domain contains a large number of charged amino acid residues that cooperate with the N domain in the conversion of the Sup35 protein (Liu et al. 2002) (Fig. 2). It is worth mentioning that the C domain maintains its biological

function in translation termination, notwithstanding the removal of domains N and M (Ter-Avanesyan et al. 1993). For this reason, domains N and M are believed to be responsible for forcing the Sup35p to behave as a prion.

Characteristics of the prion-forming domain

The prion-forming domain of the Sup35p is formed by residues 1–123. It has been analyzed using various approaches, including the overexpression of the N domain in yeast cells (Derkatch et al. 1996; Patino et al. 1996; Kochneva-Pervukhova et al. 1998). In such cells the Sup35 protein was observed to form aggregates found in [*PSI*+] cells. The PrD was further ramified into two regions: NQ (residues 1–41) and NR (residues 42–123). The former, responsible for the alteration of protein three-dimensional structure, is rich in asparagine and glutamine (N domain Q-rich), while the latter contains five oligopeptide repeats (N domain repeat) that are necessary for the propagation and transmission of aggregated Sup35p to daughter cells (Hara et al. 2003; Shkundina et al. 2006). The mechanism responsible for the stable inheritance of the yeast prion is not well understood. However, many studies have shown that the NR region can be linked with a fragmentation of the aggregated Sup35p by the heat shock protein Hsp104 (Osherovich et al. 2004).

The physiological role of the Hsp104 protein is to resolve stress-induced aggregates and help refolding of the protein. In the final stage of this process, aggregates can be divided into protein monomers (Parsell et al. 1994; Bösl et al. 2006). The cellular concentration of the Hsp104p is sufficient to the generation of prion seeds (the aggregated oligomeric Sup35 protein), which are effectively transferred with cytoplasm when the cell divides. It is the reason why all daughter cells have the [*PSI*+] phenotype (Kryndushkin et al. 2003; True 2006).

An interesting observation was that both overexpression and low level of the Hsp104 protein cure yeast cells and recover the [*psi*–] phenotype (Chernoff et al. 1995; Chernoff 2007). One of the possible explanations is that a sufficient number of prion seeds can be produced only when the appropriate number of the Hsp104 protein is present. The low concentration of the Hsp104p is not sufficient for the generation of prion seeds, and the large Sup35p aggregate is considered to be inherited by only one of daughter cells after cell division. Under a high concentration of the chaperone, the Sup35p aggregates are resolved too efficiently and become soluble in cytoplasm, leading to the disappearance of the prion seeds and reversion to the [*psi*–] phenotype. Recently, it was reported that the effect of the Hsp104 protein abundance can be decreased by deletion of either the gene coding for one of the major yeast ubiquitin-conjugating enzymes, Ubc4, or the gene coding for the ubiquitin-recycling enzyme, Ubp6 (Allen et al. 2007). As numerous attempts failed to detect ubiquitinated Sup35p in the yeast extracts, the mechanism of this phenomenon remains to be determined. Interestingly, although it is the case for the [*PSI*+], the overexpression of the Hsp104p does not cure phenotypic changes caused by other yeast prions (Derkatch et al. 1997; Moriyama et al. 2000).

It has been proposed that the [*PSI*+] prion arises as by-products of the reversible assembly of highly ordered complexes that protect the Sup35 protein during stress conditions. According to this hypothesis, reversible aggregates of the Sup35p are used to preserve this protein through unfavorable periods. After conditions change back to normal and regular cellular metabolism is resumed, the Sup35 protein could be easily activated (Chernoff 2007). Lately, it was reported that the Sse1 protein, the yeast orthologue of the mammalian Hsp110 and a nucleotide exchange factor for Hsp70 proteins, are responsible for de novo formation of the [*PSI*+] prion in *S. cerevisiae* (Fan et al. 2007). Taken together, these facts strongly suggest the involvement of stress-related proteins in prion appearance, maintenance, and propagation.

Role of the conserved region in prion protein

The presence of the PrD in the Sup35 protein may be considered as hazardous rather than profitable for its influence on the appearance of the [*PSI*+] phenotype, which makes it impossible to stop the translation at stop codons. The survey of the proteins that have similar protein sequence from other yeast species can explain the paradox. The collected data suggest that there are many examples of proteins with N-terminal sequence reminding PrD of the Sup35p of *S. cerevisiae* (Kushnirov et al. 1987; Santoso et al. 2000; Nakayashiki et al. 2001). The sequence analysis of proteins found in various yeast species homologous to the Sup35p revealed that PrD (or an equivalent sequence rich in asparagine and glutamine) is a common element of these proteins (Uptain and Lindquist 2002). Moreover, experiments carried out using the Sup35p from *S. cerevisiae* showed that all mutations preventing changes of the protein conformation are localized in the PrD (DePace et al. 1998). These observations point out that the PrD is evolutionarily conserved and plays a rather important role in cellular processes; this was confirmed through experiments performed by True and Lindquist (2000) in which viability of yeast cells was examined on plates containing various toxic substances. The experiment showed higher viability of [*PSI*+] cells, which confirms the positive influence of yeast prions on its host cells.

The analysis of PrP from a variety of mammalian species also showed that its amino acid sequence is partially conserved during the evolution. However, the natural function of PrP has not been identified to date. Some results indicate that the protein has superoxide dismutase activity and interacts with copper ions. It seems to be an important feature because the homeostasis of copper ion has an impact on functions of the central nervous system. Therefore, the amount of PrP in neurons should have some connection with the development of transmissible spongiform encephalopathies (Wechselberger et al. 2002). Moreover, recent

research indicates that PrP may be associated with human long-term memory (Papassotiropoulos et al. 2005). This observation is also supported by the identification of the prion-like domain in the protein involved in long-term memory in the sea slug *Aplysia californica* (Bailey et al. 2004). Although these data suggest the relationship with functions of the central nervous system and long-term memory, the role of PrP in mammalian cells may be limited to pathological functions.

Some authors have demonstrated that yeast prions such as [*PSI*+] may also have pathological aspects. This interpretation is based on the screening of 70 wild-type yeast strains from genus *Saccharomyces*. The supposition was that prions without negative influence on cell functions should appear in all strains with the same probability and frequency. However, the result was distinct: in 11 strains the [*PIN*+] prion was identified, but none contained the [*PSI*+] prion (Nakayashiki et al. 2005). Similar results were obtained in previous studies using fewer strains: the [*PSI*+] prion was not found, whereas the [*PIN*+] prion was detected in two strains (Chernoff et al. 2000; Resende et al. 2003). The [*PIN*+] prion is associated with a protein with unknown function coded by the *RNQ1* gene, whose deletion causes no detectable changes of the phenotype (Sondheimer and Lindquist 2000). For this reason, it is believed that the Rnq1 protein unlkie the Sup35p, is not an essential protein.

Lack of yeast strains with the [*PSI*+] phenotype may indicate that the [*PSI*+] prion has negative impact on cell physiology under normal growth conditions. Therefore, it could be concluded that the [*PSI*+] prion has a net deleterious effect on its host (Nakayashiki et al. 2005). However, if yeast prions are diseases, just like transmissible spongiform encephalopathies of mammals, which are largely diseases of later life, they should appear particularly in old cultures of yeast. This hypothesis has not been positively verified by an experiment in which old cells were isolated from [*psi*–] strain and the frequency of de novo generation of the [*PSI*+] prion was measured. There is no evidence that aging of yeast increases the frequency of prion occurrence (Shewmaker and Wickner 2006). Hence, the nature of mammalian and yeast prion disorders are considered to be diametrically different. Whether yeast prions are really diseases or not, there are data supporting the hypothesis that the [*PSI*+] prion plays an active role in yeast evolution.

Role of the [*PSI*+] prion in yeast evolution

A spontaneous readthrough of stop codons caused by the aggregation and following reduction of the functional pool of the Sup35 protein facilitates appearance of longer proteins. Some experiments indicate that wild-type and [*PSI*+] cells of *S. cerevisiae* exhibit different survival rates in various media containing different carbon or nitrogen sources. This research was carried out using more than 200 types of media. It appeared in some environmental conditions that [*PSI*+] cells had higher survival rate than wild-type strains (True and Lindquist 2000). As these data suggest, it is likely that the [*PSI*+] prions help yeast cells to survive in the hazardous or changing environments.

These observations are presumably explained in light of the evolution of yeasts. The functional Sup35 protein can convert spontaneously to the prion form (True et al. 2004). In normal conditions such conversion brings neither advantage nor harm. However, sudden environmental changes that may cause frequent mutations (including nonsense mutations) in the yeast genome promote the [*PSI*+] phenotype, because only these cells have a chance to read through premature stop codons. As a consequence, [*PSI*+] cells begin to dominate in the population.

Spontaneous readthrough of stop codons enables the synthesis of longer proteins that cannot be found in cells with correctly working translation machinery. Hence, diverse proteins produced in [*PSI*+] cells can facilitate the appearance of the phenotype corresponding well to actual environmental conditions. Moreover, if environmental changes caused mutations in the genome (including nonsense mutations), the [*PSI*+] prion makes it possible to read through premature stop codons. As a consequence of this process, the synthesis of proteins of the correct length is enabled. As the population grows, the frequency of the spontaneous conversion of the phenotype grows. [*PSI*+] cells convert to [*psi*–] cells with the rate of 10^{-6}–10^{-8} (Liu and Lindquist 1999). Such spontaneous processes are easily noticeable when the population size is large. [*PSI*+] cells are not favored by the sudden change of environmental conditions similar to the original ones. These conditions favor [*psi*–] cells, whereas [*PSI*+] cells tend to be underrepresented (Crist and Nakamura 2006) (Fig. 3).

This mechanism ensures a quick accommodation of yeast cells to unstable environmental conditions. In contrast to genetic changes, the conversion between [*psi*–] and [*PSI*+] phenotypes is rapid and reversible and, importantly, lowers the risk and cost of the evolution. The earlier work was also supporting the idea of advantageous influence of the [*PSI*+] prion on yeast cells. According to those results, the [*PSI*+] phenotype enhances thermo- and chemotolerance, although this phenomenon is not observed in all *S. cerevisiae* strains that have been examined (Eaglestone et al. 1999).

If prion proteins are actually beneficial to yeast cells, there may be some other yeast prions yet to be discovered. This assumption is based on the fact that the fragment of the *SUP35* gene coding N and M domains fused to the gene coding for the unrelated transcription factor resulted in the synthesis of the protein with two stable conformations (Li and Lindquist 2000). This result clearly shows the possibility of the de novo appearance of prions made of protein that have never been considered as a prion. Hence, natural transfer and successful recombination of the DNA fragment coding PrD to other genes can also, theoretically, lead to the production of new prion proteins.

Continuing research on the [*PSI*+] prion suggests that prion formation may engender mechanisms to uncover hidden genetic variation. Recently, the evolution of the prion-determinant domains in 21 fungi has been analyzed, focusing on compositional biases, repeats, and substitution rates. This research revealed that the region rich in aspara-

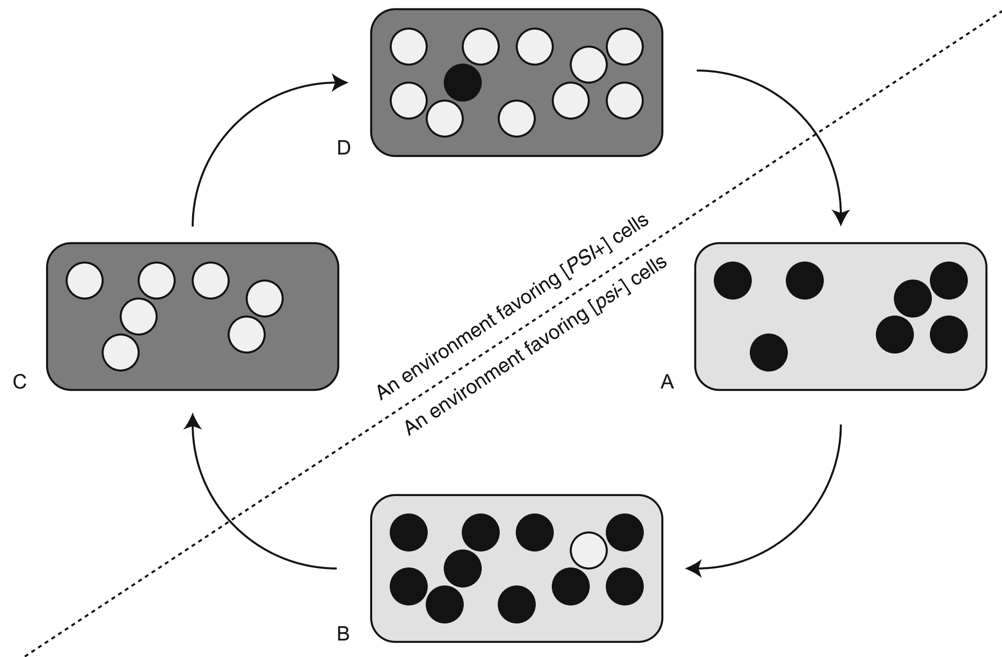

Fig. 3. Yeast evolution and influence of phenotypes caused by alternative conformations of the Sup35p. *Solid and open circles* symbolize [*psi*−] and [*PSI*+] cells, respectively. **A** An environment favoring [*psi*−] cells enables a significant growth of the population size. **B** As the population grows, it is more likely to find the [*PSI*+] cell because of the spontaneous conversion of the Sup35p structure. **C** There is a chance that [*PSI*+] cells will be better accommodated to a new environment after the change of conditions. **D** If particular environmental conditions remain stable, [*PSI*+] cells will start to dominate in the population. As the population grows, it is more likely to find the [*psi*−] cell because of the spontaneous conversion of the Sup35p structure. Another change of the environment and a return to the original conditions enable better accommodation of wild-type cells

gine and/or glutamine in the [*PSI*+] prion is maintained in fungal clades that diverged 1 billion years ago, with purifying selection observed within the *Saccharomyces* species. Among species with the highest content of asparagine or glutamine, there are *Saccharomyces castellii*, *Candida glabrata*, and *Candida albicans* (Harrison et al. 2007). The last has been shown experimentally to form prions in its own cells (Tanaka et al. 2005). Although the [*PSI*+] prion has been identified in *S. cerevisiae* and *C. albicans*, the species barrier prevents cross-species prion propagation between the two yeast species (Santoso et al. 2000). Similar species barrier exists between *S. cerevisiae* and *Pichia methanolica* (Kushnirov et al. 2000). Recent data, however, have shown that the transmission barrier can be traversed by chimeric Sup35 protein with critical sequences from *S. cerevisiae* and *C. albicans*. Results indicate that residues 9–39 of the *S. cerevisiae* Sup35p and residues 59–86 of the *C. albicans* Sup35p are responsible for the species-specific formation of self-perpetuating conformations of this protein (Tessier and Lindquist 2007).

At approximately the same time, the species barrier problem in prion transmission among three *Saccharomyces* species was investigated. As it is well known, the species barrier can be overcome, for example, in case of cattle and humans (Prusiner 1998). To better understand the issue of species barrier, regions of yeast prion protein Sup35 of *S. bayanus*, *S. cerevisiae*, and *S. paradoxus* exhibiting the range of prion domain divergence equivalent to the range of divergence observed among distant mammalian species were investigated. The research indicated that the species barrier is present in some combinations of species (asymmetrical also). The data also suggest that species specificity of prion transmission is governed at the level of conformational conversion rather than by coaggregation of prion proteins (Chen et al. 2007). Previously, the species barrier was considered to occur because of the inability of various prion proteins to coaggregate (Santoso et al. 2000).

It is a good example of the yeast-based research that brought new insight into the mechanism of prion transmission, not only between various yeast species, but also between mammals, as the mechanism of prion propagation might be universal. The continuing research should bring new insight to the mechanism of prion transmission.

The [*PSI*+] prion in screening for anti-prion drugs

The [*PSI*+] phenotype and the characteristic of each known prion – the ability of the conversion – have been successfully implemented to the screening of anti-prion drugs that can be used in the treatment of Creutzfeldt–Jakob disease. To build up an efficient screening system, researchers decided to use a well-characterized mutation in the *ADE1* gene coding for an enzyme involved in the biosynthesis of adenine. The *ade1-14* mutation is nothing but the premature stop codon in the *ADE1* gene that makes it impossible to synthesize the full-length Ade1 protein in normal cells.

The shorter Ade1p lacks enzymatic activity and enhances the accumulation of the red-colored derivative of the adenine biosynthesis intermediate in vacuoles. As a consequence, colonies produced by *ade1-14* cells are colored in red.

How is this mutation affected by the appearance of the [*PSI*+] prion in the cell? As already mentioned, in [*PSI*+] cells the Sup35 protein does not work efficiently because of its aggregation. For this reason, translation is not terminated at the proper position, enabling readthrough of the stop codon introduced by the *ade1-14* mutation, which possibly leads to the synthesis of the full-length Ade1 protein. This phenomenon can be monitored by the color of colonies. If the *ade1-14* mutation is suppressed by the [*PSI*+] prion, colonies are white. On the other hand, if the [*PSI*+] prion is cured, colonies formed by *ade1-14* cells with the [*psi*–] phenotype should be red. This mechanism is used in the analysis of chemical compounds that may cure prions (Liu and Lindquist 1999).

Taking advantage of color change of colonies, Tribouillard et al. (2006) has constructed the screening system of drugs that cure the [*PSI*+] prion. In this system, filter papers impregnated with solutions containing putative anti-prion drugs are placed on the plate covered with *ade1-14* cells containing the [*PSI*+] prion. The surface of the plate is white, as the premature stop codon in *ade1-14* locus is not working because of the [*PSI*+] phenotype. When the chemical compound diffusing from the filter paper disks interacts with the Sup35p and forces it to convert into the native structure, the protein complex responsible for translation termination begins to recognize all stop codons correctly. In this situation, *ade1-14* cells change in color to red (Fig. 4).

To date some chemical compounds curing prions, i.e., changing the pathological structure of prions into the normal one, have been characterized. The best studied example of such chemical agents is guanidine-HCl (GuHCl), which denaturates and facilitates refolding of proteins including the Sup35p in prion form (Tuite et al. 1981; Tribouillard et al. 2006). It is worth mentioning that GuHCl cures both mammalian and yeast prions. The anti-prion potential of GuHCl was also confirmed using the screening system described here; however, the mechanism of its action remains unclear (Eaglestone et al. 2000).

Examples of chemical compounds curing mammalian prions ex vivo include quinacrine and chlorpromazine, both positively verified in the yeast-based screening assay. Among newly recognized classes of molecules one can find

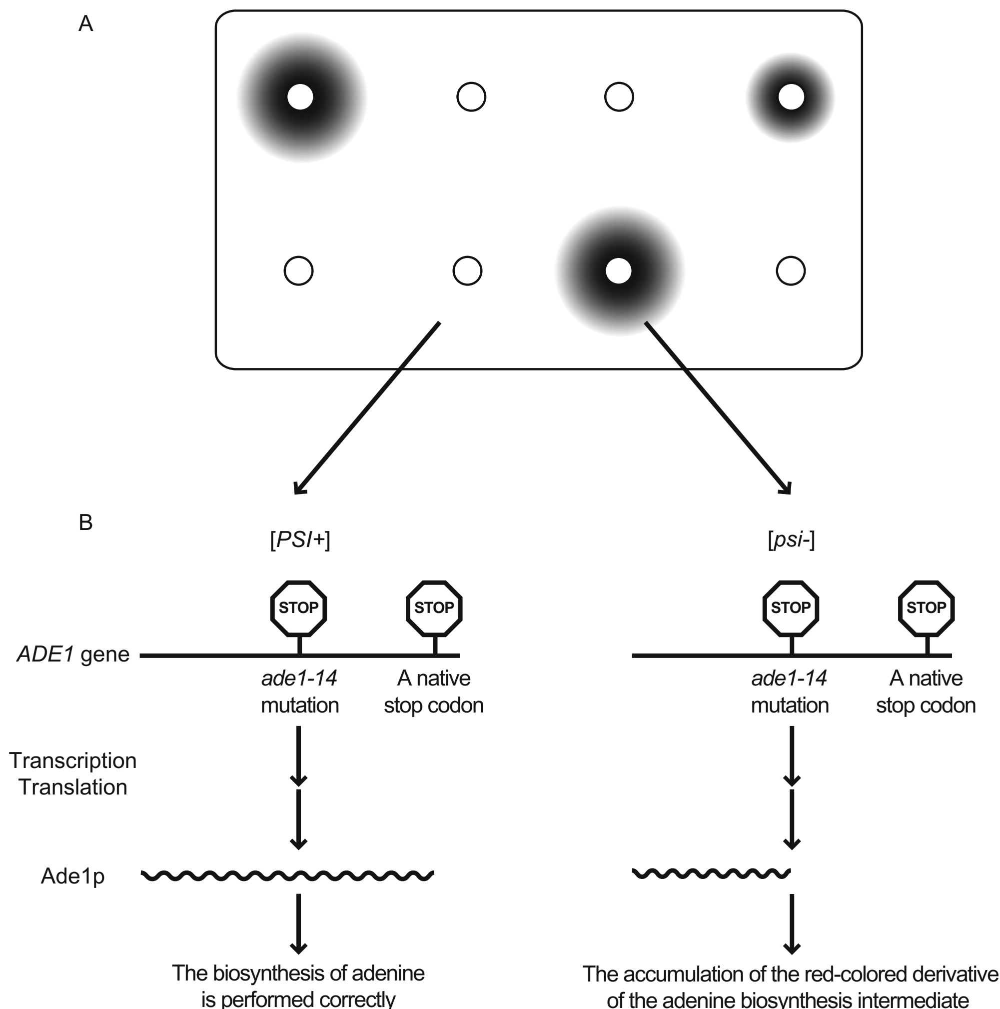

Fig. 4. The yeast-based screening system of anti-prion drugs. **A** The surface of the plate is covered with [*PSI*+] cells carrying the *ade1-14* mutation. *Open circles* illustrate filter paper disks that contain putative anti-prion chemical compound. *Dark areas* around some filter papers show yeast cells that have been cured (these areas correspond to red [*psi*–] cells). **B** Processes taking place in both types of cells. In [*PSI*+] cells, some stop codons can be read through (*left side*), while in [*psi*–] cells translation is terminated at the first (premature) stop codon in the open reading frame (*right side*)

kastellpaolitines and phenanthridine and its derivatives, in particular, 6-aminophenanthridine (Bach et al. 2003). All the molecules mentioned above were also found to be active against mammalian prion in various cellular systems (Nishida et al. 2000; Korth et al. 2001; Vilette et al. 2001). The yeast-based system allows high-throughput screening of potential anti-prion therapeutics in a simple, economic, and safe way without need for a highly secured laboratory (Tribouillard et al. 2006). Thanks to these advantages, the yeast-based screening methods are becoming more and more popular in preliminary analysis of potential anti-prion drugs.

Recently, it has been reported that the aggregation of the A beta peptides responsible for Alzheimer's disease can be studied in vivo using the yeast-based system (von der Haar et al. 2007). Hence, it is very likely that yeast-based methods will be broadly used for studying the pathogenesis of diseases in which protein misfolding and aggregation is implicated.

Conclusions

Although mechanisms of the pathogenesis and inheritance of Creutzfeldt–Jacob disease are well characterized, the physiological functions of the mammalian PrP have not been determined. However, research carried out using *S. cerevisiae* showed that prion proteins are also present in the single-celled organism and may be involved in physiological and evolutionary processes. In the case of the [*PSI*+] prion in yeast, this seems to be associated with the ability to read through stop codons introduced by nonsense mutations. This feature is valuable only in certain conditions; however, it may be especially crucial for the survival of yeast cells in environmental conditions causing frequent mutations. Moreover, there are other proteins containing the PrD that enable the conversion of the three-dimensional structure of the protein. This observation may suggest that a variety of prion proteins in the yeast *S. cerevisiae* play important roles in the cell. Nevertheless, some authors have reported that yeast prions only reflect the pathological state of the yeast cell. Which hypothesis explain the reality better? What is the sense of the PrP in mammalian cells? Intensive research carried out all over the world should bring answers to these questions.

References

Allen KD, Chernova TA, Tennant EP, Wilkinson KD, Chernoff YO (2007) Effects of ubiquitin system alterations on the formation and loss of a yeast prion. J Biol Chem 282:3004–3013

Bach S, Talarek N, Andrieu T, Vierfond JM, Mettey Y, Galons H, Dormont D, Meijer L, Cullin C, Blondel M (2003) Isolation of drugs active against mammalian prions using a yeast-based screening assay. Nat Biotechnol 21:1075–1081

Bailey CH, Kandel ER, Si K (2004) The persistence of long-term memory; a molecular approach to self-sustaining changes in learning-induced synaptic growth. Neuron 44:49–57

Benkemoun L, Saupe SJ (2006) Prion proteins as genetic material in fungi. Fungal Genet Biol 43:789–803

Bösl B, Grimminger V, Walter S (2006) The molecular chaperone Hsp104: a molecular machine for protein disaggregation. J Struct Biol 156:139–148

Callahan MA, Xiong L, Caughey B (2001) Reversibility of scrapie-associated prion protein aggregation. J Biol Chem 276:28022–28028

Chen B, Newnam GP, Chernoff YO (2007) Prion species barrier between the closely related yeast proteins is detected despite coaggregation. Proc Natl Acad Sci U S A 104:2791–2796

Chernoff YO (2007) Stress and prions: lessons from the yeast model. FEBS Lett 581:3695–3701

Chernoff YO, Derkach IL, Inge-Vechtomov SG (1993) Multicopy *SUP35* gene induces *de novo* appearance of psi-like factors in the yeast *Saccharomyces cerevisiae*. Curr Genet 24:268–270

Chernoff YO, Lindquist SL, Ono B, Inge-Vechtomov SG, Liebman SW (1995) Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [*psi*+]. Science 268:880–884

Chernoff YO, Galkin AP, Lewitin E, Chernova TA, Newnam GP, Belenkiy SM (2000) Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein. Mol Microbiol 35:865–876

Cox B (1965) [*PSI*], a cytoplasmic suppressor of super-suppression in yeast. Heredity 20:505–521

Cox B, Tuite MF, McLaughlin CS (1988) The psi factor of yeast: a problem in inheritance. Yeast 4:159–178

Crist CG, Nakamura Y (2006) Cross-talk between RNA and prions. J Biochem (Tokyo) 140:167–173

DePace AH, Santoso A, Hillner P, Weissman JS (1998) A critical role for amino-terminal glutamine/asparagine repeats in the formation and propagation of a yeast prion. Cell 93:1241–1252

Derkatch IL, Chernoff YO, Kushnirov VV, Inge-Vechtomov SG, Liebman SW (1996) Genesis and variability of [*PSI*] prion factors in *Saccharomyces cerevisiae*. Genetics 144:1375–1386

Derkatch IL, Bradley ME, Zhou P, Chernoff YO, Liebman SW (1997) Genetic and environmental factors affecting the *de novo* appearance of the [*PSI*+] prion in *Saccharomyces cerevisiae*. Genetics 147: 507–519

Doel SM, McCready SJ, Nierras CR, Cox BS (1994) The dominant *PNM2*$^{-}$ mutation which eliminates the ψ factor of *Saccharomyces cerevisiae* is the result of a missense mutation in the *SUP35* gene. Genetics 137:659–670

Eaglestone SS, Cox BS, Tuite MF (1999) Translation termination efficiency can be regulated in *Saccharomyces cerevisiae* by environmental stress through a prion-mediated mechanism. EMBO J 18:1974–1981

Eaglestone SS, Ruddock LW, Cox BS, Tuite MF (2000) Guanidine hydrochloride blocks a critical step in the propagation of the prion-like determinant [*PSI*(+)] of *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 97:240–244

Fan Q, Park KW, Du Z, Morano KA, Li L (2007) The role of Sse1 in the *de novo* formation and variant determination of the [*PSI*+] prion. Genetics 177:1583–1593

Hara H, Nakayashiki T, Crist CG, Nakamura Y (2003) Prion domain interaction responsible for species discrimination in yeast [*PSI*+] transmission. Genes Cells 8:925–939

Harrison LB, Yu Z, Stajich JE, Dietrich FS, Harrison PM (2007) Evolution of budding yeast prion-determinant sequences across diverse fungi. J Mol Biol 368:273–282

Kochneva-Pervukhova NV, Paushkin SV, Kushnirov VV, Cox BS, Tuite MF, Ter-Avanesyan MD (1998) Mechanism of inhibition of Psi+ prion determinant propagation by a mutation of the N-terminus of the yeast Sup35 protein. EMBO J 17:5805–5810

Korth C, May B, Cohen FE, Prusiner SB (2001) Acridine and phenothiazine deriverates as pharmacotherapeutics for prion disease. Proc Natl Acad Sci U S A 98:9836–9841

Kryndushkin DS, Alexandrov IM, Ter-Avanesyan MD, Kushnirov VV (2003) Yeast [*PSI*+] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. J Biol Chem 278:49636–49643

Kushnirov VV, Ter-Avanesyan MD, Surguchov AP, Smirnov VN, Inge-Vechtomov SG (1987) Localization of possible functional domains in sup2 gene product of the yeast *Saccharomyces cerevisiae*. FEBS Lett 215:257–260

Kushnirov VV, Ter-Avanesyan MD, Telckov MV, Surguchov AP, Smirnov VN, Inge-Vechtomov SG (1988) Nucleotide sequence of

the *SUP2* (*SUP35*) gene of *Saccharomyces cerevisiae*. Gene (Amst) 66:45–54

Kushnirov VV, Kochneva-Pervukhova NV, Chechenova MB, Frolova NS, Ter-Avanesian MD (2000) Prion properties of the Sup35 protein of yeast *Pichia methanolica*. EMBO J 19:324–331

Li L, Lindquist S (2000) Creating a protein-based element of inheritance. Science 287:661–664

Liu JJ, Lindquist S (1999) Oligopeptide-repeat expansions modulate "protein-only" inheritance in yeast. Nature (Lond) 400:573–576

Moriyama H, Edskes HK, Wickner RB (2000) [*URE3*] prion propagation in *Saccharomyces cerevisiae*: requirement for chaperone Hsp104 and curing by overexpressed chaperone Ydj1p. Mol Cell Biol 20:8916–8922

Nakayashiki T, Ebihara K, Bannai H, Nakamura Y (2001) Yeast [*PSI*+] "prions" that are crosstransmissible and susceptible beyond a species barrier through a quasi-prion state. Mol Cell 7:1121–1130

Nakayashiki T, Kurtzman CP, Edskes HK, Wickner RB (2005) Yeast prions [*URE3*] and [*PSI*+] are diseases. Proc Natl Acad Sci U S A 102:10575–10580

Nishida N, Harris DA, Vilette D, Laude H, Frobert Y, Grassi J, Casanova D, Milhavet O, Lehmann S (2000) Successful transmission of three mouse-adapted scrapie strains to murine neuroblastoma cell lines overexpressing wild-type mouse prion protein. J Virol 74: 320–325

Osherovich LZ, Cox BS, Tuite MF, Weissman JS (2004) Dissection and design of yeast prions. PLoS Biol 2:442–451

Papassotiropoulos A, Wollmer MA, Aguzzi A, Hock C, Nitsch RM, de Quervain DJ (2005) The prion gene is associated with human long-term memory. Hum Mol Genet 14:2241–2246

Parsell DA, Kowal AS, Singer MA, Lindquist S (1994) Protein disaggregation mediated by heat-shock protein Hsp104. Nature (Lond) 372:475–478

Patino MM, Liu JJ, Glover JR, Lindquist S (1996) Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. Science 273:622–626

Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. Science 216:136–144

Prusiner SB (1994) Biology and genetics of prion diseases. Annu Rev Microbiol 48:655–686

Prusiner SB (1998) Prions. Proc Natl Acad Sci U S A 95: 13363–13383

Resende CG, Outeiro TF, Sands L, Lindquist S, Tuite MF (2003) Prion protein gene polymorphisms in *Saccharomyces cerevisiae*. Mol Microbiol 49:1005–1017

Santoso A, Chien P, Osherovich LZ, Weissman JS (2000) Molecular basis of a yeast prion species barrier. Cell 100:277–288

Serio TR, Lindquist SL (1999) [*PSI*+]: an epigenetic modulator of translation termination efficiency. Annu Rev Cell Dev Biol 15:661–703

Serio TR, Lindquist SL (2000) Protein-only inheritance in yeast: something to get [*PSI*+]-ched about. Trends Cell Biol 10:98–105

Shewmaker F, Wickner RB (2006) Ageing in yeast does not enhance prion generation. Yeast 23:1123–1128

Shkundina IS, Kushnirov VV, Tuite MF, Ter-Avanesyan MD (2006) The role of the N-terminal oligopeptide repeats of the yeast Sup35 prion protein in propagation and transmission of prion variants. Genetics 172:827–835

Sondheimer N, Lindquist S (2000) Rnq1: an epigenetic modifier of protein function in yeast. Mol Cell 5:163–172

Tanaka M, Chien P, Yonekura K, Weissman JS (2005) Mechanism of cross-species prion transmission: an infectious conformation compatible with two highly divergent yeast prion proteins. Cell 121:49–62

Ter-Avanesyan MD, Kushnirov VV, Dagkesamanskaya AR, Didichenko SA, Chernoff YO, Inge-Vechtomov SG, Smirnov VN (1993) Deletion analysis of the *SUP35* gene of the yeast *Saccharomyces cerevisiae* reveals two non-overlapping functional regions in the encoded protein. Mol Microbiol 7:683–692

Ter-Avanesyan MD, Dagkesamanskaya AR, Kushnirov VV, Smirnov VN (1994) The *SUP35* omnipotent suppressor gene is involved in the maintenance of the non-Mendelian determinant [*psi*+] in the yeast *Saccharomyces cerevisiae*. Genetics 137:671–676

Tessier PM, Lindquist S (2007) Prion recognition elements govern nucleation, strain specificity and species barriers. Nature (Lond) 447:556–561

Tribouillard D, Bach S, Gug F, Desban N, Beringue V, Andrieu T, Dormont D, Galons H, Laude H, Vilette D, Blondel M (2006) Using budding yeast to screen for anti-prion drugs. Biotechnol J 1:58–67

True HL (2006) The battle of the fold: chaperones take on prions. Trends Genet 22:110–117

True HL, Lindquist SL (2000) A yeast prion provides a mechanism for genetic variation and phenotypic diversity. Nature (Lond) 407:477–483

True HL, Berlin I, Lindquist SL (2004) Epigenetic regulation of translation reveals hidden genetic variation to produce complex traits. Nature (Lond) 431:184–187

Tuite MF, Mundy CR, Cox BS (1981) Agents that cause a high frequency of genetic change from [*psi*+] to [*psi*–] in *Saccharomyces cerevisiae*. Genetics 98:691–711

Tuite MF, Lund PM, Futcher AB, Dobson MJ, Cox BS, McLaughlin CS (1982) Relationship of the [*psi*] factor with other plasmids of *Saccharomyces cerevisiae*. Plasmid 8:103–111

Uptain SM, Lindquist S (2002) Prions as protein-based genetic elements. Annu Rev Microbiol 56:703–741

Vilette D, Andreoletti O, Archer F, Madelaine MF, Vilotte JL, Lehmann S, Laude H (2001) *Ex vivo* propagation of infectious sheep scrapie agent in heterologous epithelial cells expressing ovine prion protein. Proc Natl Acad Sci U S A 98:4055–4059

von der Haar T, Jossé L, Wright P, Zenthon J, Tuite MF (2007) Development of a novel yeast cell-based system for studying the aggregation of Alzheimer's disease-associated A beta peptides *in vivo*. Neurodegener Dis 4:136–147

von der Haar T, Tuite MF (2007) Regulated translational bypass of stop codons in yeast. Trends Microbiol 15:78–86

Wechselberger C, Wurm S, Pfarr W, Höglinger O (2002) The physiological functions of prion protein. Exp Cell Res 281:1–8

Wickner RB (1994) [*URE3*] as an altered Ure2 protein: evidence for a prion analog in *Saccharomyces cerevisiae.* Science 264:566–569

Young CS, Cox BS (1971) Extrachromosomal elements in a supersuppression system of yeast. I. A nuclear gene controlling the inheritance of the extrachromosomal elements. Heredity 26:413–522

Young CS, Cox BS (1972) Extrachromosomal elements in a supersuppression system of yeast. II. Relations with other extrachromosomal elements. Heredity 28:189–199