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REVIEW

Prions: Infectious Proteins with Genetic Properties

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Abstract—The data on prions—proteinaceous infectious agents—are briefly summarized. Prions cause several incurable neurodegenerative diseases in mammals, while in lower eukaryotes the prion properties of proteins may be responsible for the inheritance of some phenotypic traits. The novel experimental models for finding and studying proteins with prion properties based on the yeast *Saccharomyces cerevisiae* and the fungus *Podospora anserina* are described. The significance of the prion phenomenon for biology and medicine is discussed.

Key words: protein–protein interactions, neurodegenerative diseases, non-chromosomally inherited determinants, prions, epigenetic inheritance, *Podospora anserina*, *Saccharomyces cerevisiae*

HUMAN AND ANIMAL DISEASES CAUSED BY PRIONS

Prions are a special class of infectious agents causing incurable neurodegenerative diseases in humans and animals. They differ from other pathogenic agents (viruses, bacteria, etc.) by the absence of any DNA or RNA genome, while their infectivity is related to protein. The unique feature of the prion diseases is that besides infectious transfer, they may be inherited or appear sporadically, these ways being combined with infectious transfer.

The clinical phenomenon of the prion diseases has been known for a long time, but their nature was discovered only about 15 years ago, when a prion protein was described and the term "prion" was introduced [1, 2]. Four human diseases caused by prions are known at present. They are usually characterized by a long incubation period lasting for years or even decades and are rather rare, about one case per million in the human population per year. However, one of the prion diseases, kuru, described for the natives of New Guinea and probably related to ritual cannibalism was epidemic at the beginning of the 20th century. This disease gradually disappeared following the disappearance of cannibalism. Creutzfeld–Jacob disease (CJD) is primarily a sporadic disease, but some cases of its transfer through medical procedures (iatrogenic CJD) have been described. Inherited CJD, syndrome of Gerstmann–Straussler–Scheinker, and fatal familial insomnia are dominantly inherited prion diseases related to the mutations of the prion gene [3, 4].

The interest in prion diseases has sharply increased recently in relation to the epidemic of bovine transmissible spongiform encephalopathy ("mad cow disease") in Britain, which is caused by a prion and may be transferred from cows to humans [5, 6]. At present about 40 cases of this new variant of CJD (nvCJD) have been recorded in Britain. Contrary to the ordinary CJD, this disease develops at a young age; brain pathohistology of deceased patients has shown similarity to that of the cows with spongiform encephalopathy [7]. Recently, the identity of the prion strains has been reported for humans with nvCJD and for cows with transmissible spongiform encephalopathy [8, 9]. These data show the danger of prion infection transfer to humans by consumption of meat from animals with prion diseases.

MOLECULAR MECHANISMS OF PRION INFECTION

Progress in understanding the nature of human prion diseases is related to the use of laboratory animals. According to the modern concept, the prion protein PrP^{Sc} is a special isoform of the cellular protein PrP^{C}, with poor solubility in detergents, high resistance to proteases, and propensity to aggregate. Functional differences of the two isoforms are explained by differences in their secondary structure: PrP^{Sc} has mostly β-sheet structure, while PrP^{C} is enriched in α-helical fragments. After the infectious form of the PrP protein enters an

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organism or appears spontaneously, it is able to convert the normal PrP^C into pathogenic PrP^{Sc}; this conversion being mediated by protein–protein interactions. In the framework of this concept, hereditary forms of the prion diseases may be explained by the increased ability of mutant proteins to convert into the pathogenic form.

Two models have been suggested to explain rearrangement of the PrP^C protein into the PrP^{Sc} form. According to the "heterodimer" model, the prion state is inherent for the monomer of PrP protein, and the conformational transition of the PrP^C molecule into the PrP^{Sc} form may be induced by binding to the PrP^{Sc} monomer [10]. After the PrP^C protein acquires prion properties, the dimer dissociates and two free PrP^{Sc} monomers may participate in further rounds of conformational rearrangement. This process resembles a chain reaction and can ensure rapid conversion of most PrP molecules into the prion form. Aggregation of the PrP^{Sc} molecules is considered in this model as a secondary process not related directly to the conformational conversion. The second, "polymerization", model suggests that the processes of PrP conformational conversion and aggregation are tightly related: the PrP^C protein is converted into the PrP^{Sc} form concomitantly with its binding to the PrP^{Sc} oligomer [11]. Therefore, the process resembles crystallization where the PrP^{Sc} oligomers may be considered as crystallization "seeds". Aggregates of the PrP^{Sc} protein in brain tissue of patients with prion diseases usually have filamentous or rod-like structure, which indicates its regular polymerization. The "polymerization" model is also supported by *in vitro* studies of the PrP^C conversion into PrP^{Sc} [12], which demonstrated that only high-molecular-weight aggregates, rather than monomers, of PrP possess prion-forming properties [13].

The present stage of studying the molecular basis of human and animal prion diseases started from identification of the *PRNP* gene encoding the prion protein. This gene proved to be evolutionarily conserved: it has been found in many mammals and in chicken. It was shown that the presence and the expression of this gene in both healthy and sick animals is independent from the prion infection. Though the exact function of the *PRNP* gene is not known, it was found to be inessential for viability and animals homozygous for the *PRNP* deletions have been obtained [14, 15]. The resistance of these animals to the prion infection was one of the key arguments in favor of the prion concept. On the other hand, the enhanced expression of this gene accelerated the development of the disease. All these facts are in a full accordance with the theory of the protein nature of the prions. The animals lacking the gene encoding the PrP protein are resistant to the disease just because their cells have no protein which might undergo conformational rearrangement. The increased levels of this protein should accelerate its conversion into the pathogenic form.

An important feature of prions as infectious agents is the existence of barriers for their transfer between different species despite the primary structure of PrP showing little variation in different mammalian species [2, 16]. However, in most cases these barriers do not prevent, but just slow down the infection transfer between the species. These barriers are related to the differences in primary PrP structure: hamster prions are not efficiently transferred to mice, but are well transferred to the transgenic mice expressing hamster PrP. Permeability of the barriers between species indicates the possibility of transferring animal prions to humans.

At present no method for treating prion diseases is known. However, the development of efficient therapy for prion diseases may be considered as a problem of primary importance because some predictions do not exclude the possibility of an nvCJD epidemic in the next 10-15 years [17]. Progress in therapeutic methods undoubtedly depend on our knowledge of the properties of prion proteins. The data available now already allow one to discuss some possible approaches. Promising ways to treat prion diseases may be lowering the expression level of the gene encoding the PrP^C protein, the prevention of PrP^C conversion into PrP^{Sc}, or activation of factors destroying the PrP^{Sc} aggregates. Studies using laboratory animals or simpler and more convenient experimental systems based on yeast or other lower eukaryotes may have an important role in developing methods to cure prion diseases.

MOLECULAR MECHANISMS OF "PROTEIN" INHERITANCE: PRIONS OF LOWER EUKARYOTES

The development of prion theory allowed the discovery of prion-like proteins in the yeast *Saccharomyces* and in the fungus *Podospora*. In turn, discovery of prions in lower eukaryotes contributed to the prion problem in general, demonstrating that prion phenomenon is of general biological importance, not just an exotic case.

Cytoplasmic determinants of *Saccharomyces cerevisiae* and *Podospora anserina*. The non-chromosomally inherited determinants, [PSI^+] and [*URE3*] have been described in the yeast *Saccharomyces cerevisiae.* In contrast to other yeast cytoplasmic determinants, all attempts to relate these factors to any nucleic acid have failed. Recent interest in [PSI^+] and [*URE3*] is due to a hypothesis that their unusual properties are related to the prion-like state of some yeast proteins.

The [PSI^+] factor was described as a cytoplasmically inherited determinant enhancing the effect of the weak nonsense suppressor *SUQ5* (it encodes serine tRNA with anticodon complementary to the UAA nonsense codon).

It was possible to "cure" the yeast cells from the [*PSI*$^+$] factor using low concentrations of guanidine hydrochloride (GuHCl), which induces no mutations in nuclear genes, but at high concentrations is able to cause protein denaturing. A similar effect was described for some stress factors: high temperature, high osmotic pressure, etc. At the same time, conventional mutagens had very low efficiency against [*PSI*$^+$]. The yeast strains lacking [*PSI*$^+$] factor ([*psi*$^-$] strains) were able to revert spontaneously to the [*PSI*$^+$] state with low frequency [18]. The [*PSI*$^+$] generation increased many times upon overexpression of the *SUP35* gene [19], which encodes the translation termination factor eRF3, also called Sup35p [20, 21]. It is important to note that mutations in the *SUP35* gene were phenotypically similar to the [*PSI*$^+$] determinant, being able to suppress all three types of nonsense mutations [22]. Maintenance of the determinant also depended on the *SUP35* gene: the deletions of its 5′-terminal region caused elimination of [*PSI*$^+$] [23]. The Sup35p was shown to contain two functionally distinct domains: N domain (the sequence of first 123 amino acids) and C domain (amino acids 254-685). The N domain is responsible for the maintenance of [*PSI*$^+$], but is inessential for viability. The evolutionarily conserved C domain is essential for cell viability and responsible for the function of Sup35p in translation, but unimportant for the maintenance of [*PSI*$^+$] [23-25].

The [*URE3*] determinant was discovered in the study of yeast mutants able to utilize ureidosuccinate in the presence of ammonium ions, which usually repress the uptake of ureidosuccinate. These mutants were divided in two classes: (1) recessive mutations in the chromosomal *URE2* gene encoding the repressor of the enzymes involved in nitrogen metabolism and (2) related to the appearance of the dominant cytoplasmically inherited determinant called [*URE3*]. It is interesting to note that though the phenotype of the cells containing [*URE3*] is identical to that of the *ure2* mutants, this gene is essential for the existence of the [*URE3*] determinant: deletion of the *URE2* gene, which is not an essential gene, caused the loss of [*URE3*] ([*ure3*] strain) [26]. At last, similar to the [*PSI*$^+$], the [*ure3*] strains could sometimes revert spontaneously to the [*URE3*] state. The frequency of these reversions was two orders of magnitude higher in the presence of multicopy plasmids carrying the *URE2* gene.

Another determinant with properties resembling those of [*PSI*$^+$] and [*URE3*] was found in the filamentous fungus *Podospora anserina*. This determinant, called [*Het-s*], controls the heterokaryon formation in crosses between different *P. anserina* strains [27]. Similar to yeast [*PSI*$^+$] and [*URE3*], it is cytoplasmically inherited. Its maintenance depends on the chromosomal *Het-s* gene (the null allele of *Het-s* cannot maintain [*Het-s*]). Overexpression of the *Het-s* gene increases the frequency of appearance of [*Het-s*] cells.

Prion-like properties of the proteins Sup35p, Ure2p, and Het-s. In 1994 it was shown that the genetic properties of the [*PSI*$^+$] and [*URE3*] determinants may be explained in the framework of the prion concept [26]. The role of the corresponding prions could be played by the proteins encoded by the *SUP35* and *URE2* genes, since the maintenance of [*PSI*$^+$] depended on the *SUP35* gene, and the maintenance of [*URE3*] depended on the *URE2* gene [23, 26]. Prion-like properties of the Sup35 and Ure2 proteins were suggested basing on the similarity with some features of prion infection.

A unique property of the [*PSI*$^+$] and [*URE3*] determinants is the reversibility of their loss: they may reappear in the same cells. This observation does not fit any hypothesis of autonomous genome coding for these determinants, or requires additional suggestions. In contrast, the prion hypothesis assumes that the loss of a determinant indicates just a loss of the ability of the cell to maintain the prion conformation of the corresponding protein; the protein itself remains in the cell and may regain its prion-like properties if the loss of the determinant is not caused by mutations in the corresponding nuclear gene. The prion hypothesis also explains the non-chromosomal inheritance and dominant manifestation of [*PSI*$^+$] and [*URE3*]. Upon fusion of two yeast cells only one of which contains a determinant, the cytoplasm is mixed and prion-like conformation is conferred to all molecules of the prionogenic protein, for example Sup35p or Ure2p. At last, it becomes clear why the deletions of the *URE2* gene and of the 5′-terminal region of the *SUP35* gene cause the loss of corresponding determinants: these mutants do not synthesize proteins able to convert into the prion-like state. On the contrary, the probability of spontaneous conformational conversion into prion-like state increases with the increase of the levels of these proteins.

From the above observations, some criteria of the prion-like nature were proposed for the yeast genetic factors by R. Wickner [28]: (1) reversibility of the determinant loss; (2) increased frequency of determinant appearance *de novo* at increased cellular concentration of the corresponding protein, and (3) phenotype relationship of prion and mutation of the gene for the protein.

Obviously, the properties of the [*Het-s*] determinant of *P. anserina* fit the above criteria. Therefore, unusual genetic properties of the [*PSI*$^+$] and [*URE3*] determinants of *S. cerevisiae* and [*Het-s*] of *P. anserina* may be explained assuming the prion-like behavior of corresponding proteins. Data were obtained showing biochemical similarity of Sup35p and Ure2p with PrP and their dependence on the corresponding yeast prion determinants. It was shown that proteins Sup35p and Ure2p soluble in the biologically active state may be accumulated in the [*PSI*$^+$] and [*URE3*] cells as high-molecular-weight aggregates [29-31]; both proteins from the determinant-containing cells had enhanced resist-

ance to proteolysis though lacking, contrary to the PrP^{Sc}, a protease-resistant core. Determinant-dependent increase in resistance of the Het-s protein to proteases was shown for the case of [*Het-s*] in *P. anserina* [27]. The propensity of Sup35p to aggregate depended on the [*PSI*] determinant: the molecules of Sup35p were able to interact with each other in the [PSI^+] cells and showed no interaction in the [psi^-]. It was also shown that intact N domain is essential for Sup35p oligomerization; the same domain is important to maintain [*PSI*] in the yeast cells [30, 32].

The prion-like nature of the Sup35p protein was strongly supported by *in vitro* studies of its conversion into the prion form [33]. Mixing of the lysates of [PSI^+] and [psi^-] cells induced conversion of Sup35p from the [psi^-] lysate into aggregated protease-resistant form specific for the [PSI^+] cells. Similar experiments were performed earlier for the mammalian prions [34]. The conformational conversion *in vitro* is obviously similar for mammalian and yeast prions, but the efficiency of prion conversion was much higher for yeast Sup35p, than for mammalian PrP. Finally, prion-like properties of Sup35p from the [PSI^+] cells were confirmed by the fact that purification of this protein to homogeneity did not decrease its prion-forming ability. Though yeast Sup35 and Ure2 proteins are distinct in primary structure from mammalian PrP, they show considerable similarity in conversion to the prion isoform: the purified Sup35p and Ure2p and their N-terminal fragments *in vitro* may form fibrils with β-sheet structure typical of the prion isoform of the PrP [35-37]. No fibrillar structure of this type has yet been found in [PSI^+] and [*URE3*] cells. However, one may suggest close relation of the fibrils formed *in vitro* with prion-like state of the Sup35p and Ure2p proteins, because these fibrils could serve as seeds for the Sup35p and Ure2p polymerization: their polymerization is induced by adding some fibrils to soluble proteins.

Beside Sup35p, Ure2p, and Het-s, other proteins with prion properties should exist in lower eukaryotes. Recently, a cytoplasmically inherited determinant with antisuppressor activity to *sup35* mutation was found in yeast [38]. Another yeast non-chromosomal factor, [PIN^+], affects the frequency of [PSI^+] generation [39] (see also below). The nature of these factors remains unknown. However, their sensitivity to GuHCl treatment suggests their prion nature. It was also suggested that the prion phenomenon underlies unusual properties of some fungal non-chromosomal determinants [40].

Similarity of the prion form of Sup35p and Ure2p with mammalian amyloids. Some properties of the prion form of yeast proteins Sup35p and Ure2p indicate their principal similarity with amyloid fibrils formed by some mammalian proteins. Amyloids are fibrous protein structures accumulating in some human diseases called amyloidoses [41]. Amyloid formation was shown for about 20 proteins of variable structure and function. However, the fibrils formed by these proteins are rather similar, being about 10 nm diameter, and showing high content of β-sheet structure and characteristic birefringence in polarized light. In some, though not all prion diseases PrP^{Sc} is accumulated as amyloid aggregates. Therefore, the polymerization model of prion formation considers prions as infectious amyloids. On the contrary, according to the heterodimer model the similarity of prions and amyloid fibrils is a secondary rather than fundamental trait. The data obtained for the yeast prions favor the first model. It was shown that purified Sup35 and Ure2 proteins or their N-terminal fragments are able to form *in vitro* fibrils, possessing all features of the amyloids. The monomers of these fibrils mainly form β-sheet structure common for amyloids and for PrP^{Sc} fibrils, and show typical birefringence in polarized light [35-37]. The fibrils formed by Sup35p and Ure2p are able to catalyze polymerization of these proteins. Formation of these fibrillar structures in [PSI^+] and [*URE3*] cells was not shown, however one can suggest that they form the structural basis of the prion aggregates in yeast cells, since these Sup35p aggregates could seed polymerization of the purified Sup35 protein. Other properties of Sup35p also favor the polymerization model. For example, the prionogenic activity was demonstrated *in vitro* only by aggregated Sup35p, but not by its soluble fraction from the [PSI^+] cells [33]. This model also explains the existence of various [PSI^+] strains (see below). Therefore, properties of the yeast prions show principal similarity of prions and mammalian amyloids.

STRAINS OF THE YEAST PRION DETERMINANT [PSI^+]

Common infectious agents may have interstrain variations depending on variations in genome sequences. Prions have no genome, but nevertheless they have various strains. It was shown that different prion isolates may show different disease character (incubation periods, symptoms, pathological changes in the tissues) which are maintained even after several passages. The existence of various prion strains was confirmed biochemically. An important feature of the pathogenic form of the PrP protein is the presence of proteinase-resistant region revealed after treatment with proteinase K. It was found that in various strains of PrP^{Sc} these proteinase-resistant fragments are of various length, as seen by the differences in electrophoretic mobility after incubation with proteinase K. The differences were also reproduced *in vitro* in the reaction of conformational rearrangement of PrP, when PrP^{Sc} from various prion strains was used as a seed [42, 43]. It was shown recently that PrP^{Sc} of different strains have different confor-

mations [44]. Eight different strains of PrP^{Sc} were identified in hamster using a specially developed immunological assay [45].

Interstrain variations are common not only for the mammalian prions. Recently the existence of different [*PSI*$^+$] variants was demonstrated: [*PSI*$^+$] isolated in the same yeast strain had different efficiency of nonsense suppression [46]. It was shown that these differences are the property of the determinant itself and do not depend on genetic background of the host strain (N. V. Kochneva-Pervukhova, unpublished).

Thus, one can conclude that interstrain differences are common for various prions. The existence of the prion strains fits much better the polymerization model, rather than the heterodimer model. In fact, it appears unlikely that protein monomers may stably exist in several alternative conformations, each one being capable of autocatalytic reproduction. In the framework of the polymerization model any possible conformation is stabilized by intermolecular interactions within the polymer. Moreover, strains may differ not only in the ternary structure of prion molecules, but also in their quaternary packing. It is known that Sup35p and Ure2p can form polymer fibrils *in vitro*. The fibrils of Sup35p differed in structure and diameter, and these features were conserved within a fibril [35]. These results confirm the existence of structurally different polymers of a protein and stable transfer of its properties during polymerization.

An interesting peculiarity of the [*PSI*$^+$] interstrain differences is that the manifestation of their phenotype is correlated with mitotic stability: as a rule, the higher is suppression efficiency of a determinant, the more stable is its inheritance [46]. Suppression efficiency obviously correlates with concentration of the soluble, non-prion Sup35p. One may naturally suggest that various polymer structures of Sup35p may have different polymerization efficiency. More efficient polymerization should be related to lower concentration of soluble Sup35p and stronger suppression. At low polymerization efficiency the suppression is decreased while the probability of the [*PSI*$^+$] prion lose is increased. These assumptions were recently confirmed experimentally. It was shown that in cells with "weak" [*PSI*$^+$] aggregation of Sup35p was lower that in cells with "strong" [*PSI*$^+$]. Besides, in the cell-free system of prion conversion, Sup35p from the cells with "weak" [*PSI*$^+$] was less efficient in seeding polymerization, than the protein from cells with "strong" [*PSI*$^+$] (M. B. Chechenova, unpublished).

GENETIC FACTORS INFLUENCING THE APPEARANCE AND MAINTENANCE OF YEAST PRIONS

Various models of prion formation have a common point that the prion conversion is of autocatalytic nature and does not require any additional factors. This was confirmed by the experiments in cell-free systems. It is not excluded, however, that the appearance and maintenance of prions *in vivo* may depend on interaction with other proteins. Some authors postulate the existence of an unidentified mammalian protein able to interact with PrP protein stimulating its conformational conversion into the prion form [47]. More direct data were obtained for yeast, confirming involvement of chaperones in the maintenance of prion conformation. It was shown that the maintenance of [*PSI*$^+$] depends on the activity of a chaperone protein Hsp104p: its absence or overexpression eliminated [*PSI*$^+$] [48]. It is known that Hsp104p is essential for dissolving the aggregates of denatured proteins [49], and that it may interact with prion form of Sup35p [50]. We suggested a simple, though paradoxical explanation of the effects and role of Hsp104p [51] based on the fibrillar structure of prion Sup35p aggregates and ability of Hsp104p to disaggregate denatured proteins. The effect of Hsp104p on the prion fibrils of Sup35p may be similar to its effect on denatured proteins. For the fibrils this action should result in fragmentation at random sites. This increases the number of fibril ends, which catalyze polymerization, therefore the overall rate of polymerization increases. However, excess Hsp104p may dissolve all Sup35p aggregates eliminating [*PSI*$^+$]. Moreover, the fragmentation is essential for stable inheritance of prions, that is, for prion transfer into daughter cells at cell division, since only the fragmentation can increase the number of prion aggregates.

Besides Hsp104p, some other yeast chaperons are involved in the maintenance of [*PSI*$^+$]. Recently it was shown that overexpression of the *SSA1* gene, encoding in yeast the Hsp70p chaperone, prevents the [*PSI*$^+$] loss upon *HSP104* overexpression [52]. This observation explains why the heat shock and other stress factors which induce both Hsp104p and Hsp70p chaperones do not cause [*PSI*$^+$] loss. The influence of chaperones on the maintenance of *S. cerevisiae* [*URE3*] and *P. anserina* [*Het-s*] determinants is almost not studied. Recently it was reported that overexpression of Hsp104p inhibits the [*URE3*] phenotypic manifestation while the *HSP104* deletion does not influence the maintenance of this determinant [53].

It remains unknown whether humans and animals have a gene homologous to yeast *HSP104*. However, since mammalian prions most probably form fibrils similar to yeast prions, the fragmentation may be considered crucial for prion replication [51, 54]. Control of the factors involved in fragmentation may become a promising strategy for curing the prion diseases.

The appearance of prions and maintenance of their properties may depend not only on chaperons, but on other interacting proteins. As common for all eukaryotes, yeast eRF3 (Sup35p) interacts with nonsense-

codon-recognizing eRF1 factor (in yeast usually called Sup45p) [21, 32]. This allowed us to suggest that the interaction with Sup45p may affect the Sup35p transition into the prion from. In fact, overexpression of *SUP45* decreased the frequency of the [*PSI*$^+$] generation, though was unimportant for its maintenance [55]. Sup35p interacts with some other proteins, among those with Upf1p involved in the control of stability of nonsense-codon-containing mRNA [56]. However, contrary to Sup45p, overexpression of Upf1p had no effect on the appearance of [*PSI*$^+$] (N. V. Kochneva-Pervukhova, unpublished).

Besides [*PSI*$^+$] and [*URE3*], one more cytoplasmically inherited determinant was found in yeast that may be of prion nature [39]. This determinant called [*PIN*$^+$] has no phenotype on its own, and its presence in yeast cells may be detected only by their ability to transform from [*psi*$^-$] state into [*PSI*$^+$] state: the overexpression of full-sized Sup35p induces [*PSI*$^+$] only in the [*PIN*$^+$]-containing cells. At the same time, the presence of the [*PIN*$^+$] determinant has no effect on the [*PSI*$^+$] stability. The nature of this determinant and the mechanism of its effect on Sup35p conversion into prion state remain unknown. The effect of [*PIN*$^+$] on generation and maintenance of [*URE3*] has not been studied.

MUTATIONS IN GENES ENCODING YEAST PRION PROTEINS WHICH ARE INCOMPATIBLE WITH PRION PROPERTIES

Yeast is a convenient model to study the factors able to "cure" cells from the prions. Contrary to mammals, some of such factors are already known for lower eukaryotes. For example, [*PSI*$^+$], [*URE3*] of *S. cerevisiae* and [*Het-s*] of *P. anserina* may be removed by GuHCl or some stress factors. [*PSI*$^+$] may also be removed by altered levels of Hsp104p chaperone [30, 48]. Naturally, mutations affecting the [*PSI*$^+$] maintenance may appear in the *SUP35* gene. It is interesting that some of these mutations are dominant, that is, able to eliminate [*PSI*$^+$] in the presence of the wild-type *SUP35* allele. This means that corresponding mutant proteins may actively interfere with the Sup35p conformational rearrangement. One of the best studied mutants of this type is Sup35p with the substitution of glycine 58 for aspartic acid [57]. This mutation does not affect the binding of Sup35p molecules to the prion aggregate, but decreases the efficiency of its subsequent conformational rearrangement and/or the efficiency of binding of the next molecule of Sup35p [58].

The properties of the mutant Sup35p suggest a new strategy for the treatment of human prion diseases. This strategy may be based on a search for mutant human PrP protein possessing properties similar to those of the Sup35p mutant described above. This protein should be able to bind to PrP aggregates and prevent further polymerization.

Interesting data were obtained in the study of the effect of various Ure2p deletions on the appearance and stability of the [*URE3*] determinant. Overexpression of the C-terminally truncated Ure2p molecules was much more efficient in the [*URE3*] induction than overexpression of the entire Ure2p [59]. Nevertheless, overexpression of partially N- and C-terminally deleted Ure2p in the [*URE3*] cells resulted in loss of the determinant [31]. Binding of Ure2p molecules of variable size to the prion polymer probably affects its structural regularity and therefore decreases its prion-forming ability. In principle, these results may form a basis for a new approach to the therapy of prion diseases. In fact, it was shown recently that peptides corresponding to some PrP fragments may slow down its prion conversion *in vitro* [60].

It should be noted that the yeast [*PSI*$^+$] determinant did not disappear at overexpression of various fragments of the Sup35 protein (Ter-Avanesyan and Kochneva-Pervukhova, unpublished); this may indicate a difference in the properties of the Ure2p and Sup35p prions.

BIOLOGICAL ROLE OF THE PRIONS IN LOWER EUKARYOTES

Prions and amyloids are associated with mammalian diseases. However, in yeast and in the fungus *Podospora* the inherited triggering of protein activity by their conversion into the prion-like state is most probably of adaptive importance. The prion mechanism may have some advantages over the common mechanisms of genetic variations. Conversion of proteins into the prion-like state may occur more frequently than mutations. At the same time, as shown for the [*PSI*$^+$], the strength of the prion phenotype may vary depending on the prion strain. It should also be noted that the reverse transition from the prion state to the normal one is more frequent that reversion of mutations, because contrary to mutations the cells with the prion phenotype preserve the information about the original state of the protein. This is of particular importance for the adaptive lability of a population, because for a unicellular organism the temporary phenotype correction in response to the environmental variations is often more important that permanent phenotype change. Therefore, prion determinants produce phenotypic diversity in the cell population while preserving the information about the basic optimal phenotype to change back to it when necessary.

The advantages of the prion-like state are rather obvious for the [*URE3*], because conversion of Ure2p into the prion form allows yeast to utilize some poor nitrogen sources. On the other hand, non-prion [*ure3*]

state may be preferable if better nitrogen sources are available [26]. Some recent data suggest the biological significance of the [*PSI*$^+$] prion determinant in yeast. It was shown that at some stress conditions the prion aggregates of Sup35p are partially and reversibly dissolved, thus enhancing the efficiency of translation termination. Therefore, the prion state of the Sup35p allows "fine tuning" of the translation termination efficiency according to the environmental conditions; this may be of adaptive importance for the yeast. Moreover, the presence of the [*PSI*$^+$] determinant by itself increases the resistance of yeast cells against heat shock and chemical stress [61].

The [*Het-s*] determinant of *P. anserina* may be considered as an even better example of biological importance of prions [27]. This determinant is a key element of the system for vegetative incompatibility which prevents crosses between distantly related fungal strains. Such crosses are potentially advantageous as they increase the genetic diversity in the *P. anserina* population. However, they carry the danger of transferring viruses (contrary to the mammals, the yeast and fungal viruses are transferred only at cell fusion). The ability of the Het-s protein to undergo spontaneous and reversible conversion into the prion state makes the population of the fungal cells heterogeneous for the presence of the [*Het-s*] determinant. The cells lacking [*Het-s*] have the advantage of genetic exchange, while the cells with this determinant are protected from viruses. This double strategy combines the advantages of both states of the Het-s protein for the *P. anserina* population.

The mechanism of vegetative incompatibility in *P. anserina* remains unknown and may be rather complicated. However, one may suggest that the Het-s protein plays the role of a sensor, because the incompatibility depends on minor changes in its structure. It is important to note that prion properties of the Het-s protein allow efficient realization of this function, because the process of prion transformation is very sensitive even to the minor structural differences of the molecules involved in it.

CONCLUSIONS

For a long time all known cases of the prion phenomenon were related only to the mammalian PrP protein. Discovery of the prion-like proteins in lower eukaryotes contributed significantly to our knowledge. It has become evident that the prion phenomenon is not only principally new, but also wide-spread in nature. The studies of the yeast prion proteins confirmed the fundamental similarity of prions with amyloid fibrils formed by about 20 human proteins. Therefore, prions were found to be just a special case, though a rather distinct one, of a general biological phenomenon. Neither infectious character nor relation to diseases is characteristic of this phenomenon in general. For example, amyloids, apart from those formed by PrP, are not infectious, and the yeast prions are infectious only in a limited sense: they are transferred through the cytoplasm, but not through the intercellular space. A key point of this phenomenon is a unique mechanism of conformation transfer between the molecules of the same protein, probably realized through formation of "one-dimensional crystal" fibers. The prion-amyloid phenomenon is intensely studied at present by many laboratories, and the number of the diseases known earlier for which the amyloid nature is confirmed grows higher. For example, recently the formation of amyloid aggregates was shown for the proteins with polyglutamine fragments related to six diseases (of which Huntington's disease is the most known) [62]. The same was shown for the sinuclein protein (Parkinson's disease) [63]. The discovery of new processes based on the prion principle in higher organisms should be expected in the near future. These mechanisms may be important for cellular aging and differentiation as well as for inheritance of some phenotypic traits, similar to the prion determinants in lower eukaryotes.

As already mentioned above, the existence of prion-like proteins may be of adaptive importance for yeast and fungi. Therefore, these proteins may be widespread in lower eukaryotes. This shows the importance of the search for new prionogenic proteins in eukaryotic microorganisms, evaluating their role and estimating their number. The latest studies have revealed two new yeast determinants with properties similar to those of [*PSI*$^+$] and [*URE3*], which may be of the prion nature [38, 39]. The same nature is not excluded for some genetic factors in filamentous fungi [40].

The yeast prions appear to be a convenient model system to study the prion phenomenon. The advantages of the yeast prions over mammalian PrP are their availability and the possibility to run rapid and safe experiments. The yeast *S. cerevisiae* is well developed as a model organism for molecular genetic studies and their prions are not dangerous for man. These advantages assure rapid progress in studying yeast prions. These studies should contribute significantly to our understanding of the molecular mechanisms of protein conversion into the prion state and to our knowledge about the occurrence and biological role of the prion phenomenon in general.

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