ELSEVIER

Mutation Research 488 (2001) 39–64

Reviews in Mutation Research

www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Mutation processes at the protein level: is Lamarck back?

Yury O. Chernoff*

School of Biology and Institute for Bioengineering and Bioscience, Georgia Institute of Technology, M/C 0363, 315 Ferst Drive, Atlanta, GA 30332-0363, USA

Received 17 August 2000; received in revised form 13 October 2000; accepted 13 October 2000

Abstract

The experimental evidence accumulated for the last half of the century clearly suggests that inherited variation is not restricted to the changes in genomic sequences. The prion model, originally based on unusual transmission of certain neurodegenerative diseases in mammals, provides a molecular mechanism for the template-like reproduction of alternative protein conformations. Recent data extend this model to protein-based genetic elements in yeast and other fungi. Reproduction and transmission of yeast protein-based genetic elements is controlled by the "prion replication" machinery of the cell, composed of the protein helpers responsible for the processes of assembly and disassembly of protein structures and multiprotein complexes. Among these, the stress-related chaperones of Hsp100 and Hsp70 groups play an important role. Alterations of levels or activity of these proteins result in "mutator" or "antimutator" affects in regard to protein-based genetic elements. "Protein mutagens" have also been identified that affect formation and/or propagation of the alternative protein conformations. Prion-forming abilities appear to be conserved in evolution, despite the divergence of the corresponding amino acid sequences. Moreover, a wide variety of proteins of different origins appear to possess the ability to form amyloid-like aggregates, that in certain conditions might potentially result in prion-like switches. This suggests a possible mechanism for the inheritance of acquired traits, postulated in the Lamarckian theory of evolution. The prion model also puts in doubt the notion that cloned animals are genetically identical to their genome donors, and suggests that genome sequence would not provide a complete information about the genetic makeup of an organism.

Keywords: Prion; Amyloid; Structural inheritance; Chaperone; Protein conformation

1. "Gene" and "mutation" in classical and molecular genetics

Genes were first thought of as structural and functional units of inherited information. Alternative states of the genes (alleles) were postulated to control differences between alternative phenotypic traits. "Mutations" were thought of as any changes in phenotype, which became inherited. In particular, gene mutations were defined as changes that convert one allele into the other allele. In classical genetics, the definition of "gene" and "mutation" did not imply any specific molecular basis of the carriers of genetic information, least of all the nucleic acids (see [1–3]). Indeed, until at least the mid 1940s it was generally agreed that the carriers of genetic information are probably proteins. Protein-based inheritance was at the heart of the early models of replication of genetic material.

The "double helix" [4] and further developments have changed the paradigm. The "central dogma" of molecular biology identified the genes as pieces of nucleic acids (in most cases, DNA). The most recent textbooks (e.g. [5]) define genetics as "the study of

* Tel.: +1-404-894-1157; fax: +1-404-894-0519.
E-mail address: yc22@prism.gatech.edu (Y.O. Chernoff).

1383-5742/01/$ – see front matter
PII: S1383-5742(00)00060-0

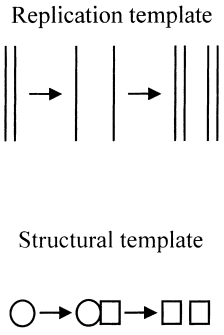


Fig. 1. Comparison of the structural and replication templates. Replication template directs synthesis of a new sequence which exactly corresponds to a pre-existing sequence. Structural template directs shaping of the new structure which exactly corresponds to a pre-existing structure.

genes" and gene as "a section of ... DNA". The term "mutation" was now assumed to apply to the changes affecting the sequence of nucleotides in DNA. While functional assays such as the complementation test remained in use, they were now held secondary to molecular evidence. The roles seemed to be assigned once and forever: genetic role — to nucleic acids, enzymatic role — to proteins. And while the discovery of enzymatic properties of RNA did shake the principle of "power separation", it had not cast any doubt in that proteins cannot be information carriers and have nothing to do with foundations of inheritance.

Such an unanimous acceptance of DNA's genetic monopoly becomes even more surprising, if we take into consideration that the first credible evidence of "non-nucleic acid" inheritance is almost as old as the double helix. So-called cortical inheritance, or cytotaxis in *Paramecium*, first described by Beisson and Sonneborn [6], was shown to be controlled by the structural organization of cortex, a multiprotein complex forming the outer surface of the ciliate cell. Surgical alterations of cortex became inherited in cell generations, indicating that pre-existing high order structure can serve as a "template" directing the formation of new structures (Fig. 1). A lot of information has been acquired that supports the "structural inheritance" model (e.g. [7]; see also [8] for review). However, all this evidence has been unable to slow down the "nucleization" of genetic terminology. Apparently, the scientific community was unprepared to recognize the universal importance of these data due to the peculiar nature of ciliates. Information obtained in such a non-conventional system could unlikely be commonly accepted as a demonstration of the general rule.

2. Prion model

The convincing proof of the genetic function of DNA has originated from experiments with infectious agents. The same is true for protein-based inheritance. The behavior of protein-based infectious agents, called "prions" by Prusiner [9], could not be explained completely within the framework of the "central dogma". Although the prion model was initially applied to infection rather than inheritance, it promoted the revolutionary idea that proteins can transmit certain information directly from one protein molecule onto another protein molecule.

Prion diseases are infectious, fatal and incurable neurodegenerative diseases in mammals, including humans. The oldest example described is scrapie disease in sheep, and the most widely publicized one is "mad cow disease", or bovine spongiform encephalopathy (BSE), which has apparently been transmitted from sheep to cows (see [10,11] for review). The human equivalents of these diseases, such as an exotic kuru disease transmitted by cannibalism, or Creutzfeldt–Jacob disease spread primarily by medical mistreatment (see [10,11]), are relatively rare, but they have gained a lot of publicity recently due to the possibility of transmission of "mad cow disease" from cows to humans through consumption of the meat of infected animals. An unusual feature of prion diseases is an extremely high resistance of the infectious agent to anti-nucleic acid treatments. After it was first reported by Alper and coworkers [12] that the scrapie agent is UV-resistant, Griffith [13] came out with a speculative model that could explain this phenomenon. The current prion concept, which took shape from the solid experimental material accumulated by Prusiner and other laboratories, has filled the Griffith's speculation with the material contents and remains the only model which is able to explain the peculiar nature of these diseases. Although the prion concept still lacks formal proof, the amount of circumstantial evidence collected was sufficient for most researchers in the field to accept it, and for the

Nobel Prize committee to award a Nobel Prize to S.B. Prusiner in 1997. A few "dissenters", remaining sound in criticizing certain aspects of the concept (for instance, see [14]), have so far failed to present an equally convincing alternative explanation.

According to the prion concept, the infectious agent is a protein in an altered conformation (called prion protein) that can reproduce itself by converting the target cellular protein of the same amino acid sequence into prion conformation. Indeed, the infectious units isolated from the brains of sick animals are primarily composed of a particular protein called PrP^{Sc} (from "scrapie"), that represents an abnormal isoform of the normal cellular protein PrP^{C} (from "cellular"). Deletion of the gene coding for PrP makes mice immune to the scrapie infection, apparently due to absence of the target, on which PrP^{Sc} could act. In the prion model, DNA remains a source of the primary material for conversion: the amino acid sequence of PrP is translated from the cellular PrP gene. However, the DNA sequence does not exclusively control a difference between phenotypic traits anymore. Both PrP^{Sc} and PrP^{C} can be expressed from one and the same nucleotide sequence, even though they cause different phenotypes. And while PrP^{C} conformation remains stable in the absence of PrP^{Sc}, it is converted into PrP^{Sc} once the PrP^{Sc} "template" is present, despite the fact that the sequence of nucleotides in the gene, as well as the sequence of amino acids in the protein, remains unchanged.

There are two principal molecular models proposed to explain the mechanism of prion conversion (see Fig. 2). The "heterodimer" model [15,16] postulates that conversion occurs in the heterodimer PrP^{C}–PrP^{Sc}, so that the shape of one partner (PrP^{C}) is changed according to the shape of the other partner (PrP^{Sc}). Since it is difficult to imagine that a stable protein conformation could be converted directly into another stable protein conformation, it is more likely that the process of conversion involves an unstable partially unfolded intermediate. The "nucleated polymerization" model [17] suggests that PrP^{Sc} is an insoluble polymer, which can "seed" polymerization of the normally soluble cellular protein (PrP^{C}), thus converting it into a prion (PrP^{Sc}). Indeed, PrP^{Sc} is insoluble and can form fiber-like polymerized aggregates, rich in β-structures and possessing characteristic features of amyloids [18,19]. While it remains to be proven whether aggregation plays a causative role in prion conversion, it is clear that molecules in prion form tend to aggregate. Certain steps of PrP^{C} aggregation "seeded" by PrP^{Sc} were reproduced in vitro [20], although numerous attempts to produce infectious material in the test tube have failed so far. As the conformation of molecules included into amyloid would necessarily be changed, compared to their soluble counterparts, it is possible that both models actually emphasize different aspects of one and the same process.

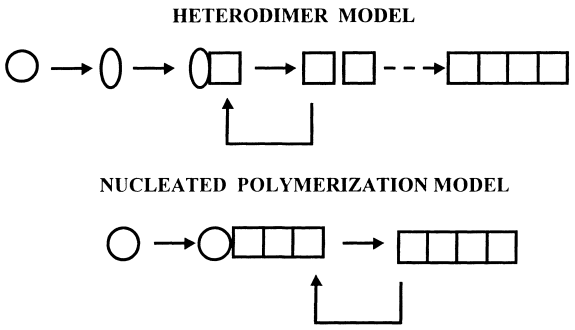


Fig. 2. Molecular models of prion propagation. (○) Cellular isoform, (⬯) partially unfolded intermediate, (□) prion isoform. See comments in the text. The "heterodimer" model is also known as "template assistance" [16] or "monomer directed conversion" [37]. For more detailed description of the models, see [37].

3. Inherited prions as genetic elements

Further extension of the prion concept has been initiated by Wickner [21], who has proposed that the *Saccharomyces cerevisiae* non-Mendelian elements [URE3] and [PSI] are prions, inherited at the cytoplasmic level. [URE3], discovered by Lacroute and coworkers [22,23], confers to the yeast *ura2* mutants the ability to utilize ureidosuccinic acid as a source of uracil. [PSI], discovered and extensively studied by Cox and coworkers [24,25], increases nonsense-suppression (i.e. readthrough of termination codons). Both elements have all the features of the "classical" non-Mendelian genes, but also require a nuclear element, *URE2* gene in case of [URE3] [21] or N-terminal domain of the *SUP35* gene in case of [PSI] [26,27], for their maintenance. Further experiments confirmed that [URE3] [28,29] and [PSI] [30,31] are associated with aggregated protease-resistant isoforms of the Ure2 and

Table 1
Comparison of the yeast and mammalian prions

	Mammalian PrP^{Sc}	Yeast [PSI]	Yeast [URE3]
Genetic features			
Transmission	Extracellular	Cytoplasmic	Cytoplasmic
Reversible curing	?	Yes	Yes
Maintenance gene	*prn-p*	*SUP35* (N-domain)	*URE2*
Phenotype of the maintenance gene mutation	Same as PrP^{Sc}	Same as [PSI]	Same as [URE3]
Induction by overexpression of the maintenance gene	?	Yes	Yes
Protein features			
Protease resistance	Yes	Yes	Yes
In vivo aggregation	Yes	Yes	Yes
In vitro amyloid formation	Yes	Yes	Yes
Protein structure			
N-terminal QN-rich stretch	No	Yes	Yes
Oligopeptide repeats	Yes	Yes	No
Unstructured N-proximal region	Yes	Yes	Yes
Protein function	Membrane-bound protein of unknown function	Translation termination factor	Regulator in nitrogen metabolism

Sup35 proteins, respectively. Similar to mammalian PrP, prion-forming domains of the Ure2 and Sup35 proteins can produce β-rich amyloid-like polymers in vitro, which can "seed" polymerization of the soluble protein of the same sequence [32–36]. At least in case of the prion-forming domain of Sup35, aggregation starts from the unstructured rather than from the completely folded protein, and proceeds via oligomeric intermediates [37]. Therefore, such a process (called "nucleated conformational conversion") combines some features of the "heterodimer" and "nucleated polymerization" models. "Seeded polymerization" of the $Sup35^{PSI+}$ aggregates was also reproduced in the yeast cell-free extracts [32,38]. Transient overproduction of either complete Ure2 or Sup35 proteins or prion-forming domains of these proteins in the yeast prion non-containing cell leads to increased de novo formation of prion isoforms of the respective proteins [21,28,39,40]. In a similar way, the liposome-mediated transfection of [psi^-] (prion non-containing) yeast cells with the prion inducing fragments of the Sup35 protein, produced and aggregated in vitro, resulted in low efficient but detectable conversion into the [PSI^+] (prion containing) state [41]. The genetic and biochemical evidence leading to discovery of yeast prions is summarized in recent reviews [42–45].

While the normal cellular function of mammalian PrP is not clear yet, functions of Ure2 and Sup35 are known (see Table 1). The Sup35 protein is a translation termination (release) factor, the yeast representative of the eukaryotic eRF3 family (see [42,44,45] for review). The Ure2 protein is performing a regulatory function in the nitrogen metabolism pathway (see [43,44] for reviews). Although PrP, Ure2 and Sup35 proteins are not homologous to each other, mammalian and yeast prions have many features in common, from phenomenology of the process to certain molecular characteristics of the prion isoforms (see Table 1). The major difference is that, in contrast to mammalian prions, [URE3] and [PSI] are transmitted by cytoplasmic transfer rather than by extracellular infection. While this generally follows the mode of transmission of yeast viruses, which are usually not capable of extracellular infection, such a mechanism establishes yeast prions as discrete genetic elements responsible for certain phenotypic traits. Formally speaking, this fits a general definition of gene in "before DNA" era (see [1–3]).

4. Prion conversion as an example of "protein mutation"

As in the case of mammalian PrP, yeast prions are not completely independent of the cellular genome, since they require the nuclear genes to code for prion

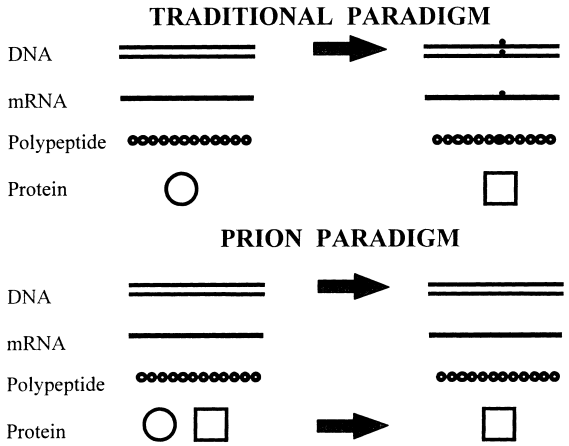


Fig. 3. Comparison of the traditional paradigm and prion paradigm. In the traditional paradigm of inheritance, the protein change becomes an inherited trait only in case if it is caused by the change in the coding DNA sequence. In the prion paradigm, one and the same sequence can take alternative shapes ("normal shape" and "prion shape"), and prion isoform could be inherited in a template-like fashion (see above, Figs. 1 and 2). Therefore, the protein change can become an inherited trait even without a corresponding change of DNA sequence.

proteins. However, differences between alternative phenotypic traits are not determined by differences in DNA sequence: both "prion containing" and "prion non-containing" cells may have one and the same sequence of nucleotides in DNA, nevertheless they exhibit alternative phenotypic traits that are inherited in cell generations (see Fig. 3). Therefore, the "cellular" or "wild-type" (non-prion) isoform and prion isoform could be considered as alternative "alleles" of the protein-based genetic element. Conversion of one allele into another would then be analogous to "mutation" at the protein level.

While use of the word "mutation" in such a context could shock a DNA-minded geneticist of modern times, this should be noted that "mutation" initially meant nothing more than "inherited change of phenotype" (see [1–3]). It would also be misleading to say that definition of mutation was specified and clarified later. Indeed, the genetic events that have very little in common except inherited change, such as polyploidy on the one hand and base substitutions on the other hand, continue to be called by one and the same name, "mutation", in the modern genetic literature (e.g. see [5]). Neither is the term "mutation" reserved strictly for DNA or genomic RNA. The recently described phenomenon of "transcriptional mutagenesis" [46,47] does not fit such a restricted mode of usage. Some researchers suggest the term "epimutation" for the inherited changes not affecting DNA sequence [48]. However, the terms "epimutations" and "epigenetics" are also associated with DNA modifications such as methylation. Therefore, application of these terms to protein-based inheritance could be a source of confusion.

The mechanism of the initial appearance of prion isoforms (the process which we suggest to call "prionization", in order to distinguish it from the propagation of pre-existing prion isoform) remains a mystery. DNA mutations in the PrP coding gene, leading to a protein with increased prion-forming potential, were shown to be associated with so-called "genetic" forms of prion diseases in mammals, including humans. By analogy, it has been suggested that prionizations could result from such DNA mutations in some somatic cells (see [10]). The mutant proteins could spread extracellularly to the wild-type cells, converting normal proteins into prions capable of further spreading the infection. However, such a mechanism is not applicable to yeast where extracellular prion infection is unlikely. Instead, it has been shown that transient overproduction of the corresponding wild-type protein [21,39] or its fragments encompassing the prion-forming domain [28,40] results in a sharp increase of prionization in yeast. This observation, which served as one of the foundation stones for the prion model of [PSI] and [URE3], also proves that initial prionization in yeast occurs at the "post-DNA" level and should be considered as protein rather than DNA "mutation". One cannot rule out a possibility that accumulation of the aberrant misfolded translation products, which initiate a process of prionization, is caused or facilitated by transcription errors or transcript alterations, such as ones described in [46,47]. However, it is also likely that spontaneous misfolding and aggregation simply become more probable with an increase of the cellular concentration of the corresponding protein. The overproduction may also shift the balance between the newly synthesized protein and chaperone helpers, assisting in proper protein folding. This might facilitate protein misfolding, resulting in prion formation (see below).

Prion isoforms are usually dominant over "wild-type" isoforms due to the ability of a prion to convert a normal protein into the prion state. This is typical of "gain-of-function" mutant alleles. Indeed, prionization confers a new function to the protein — that is, the ability to reproduce the prion state. Meanwhile, the normal cellular function of the same protein is usually impaired or lost in the prion isoform. Due to wild-type to prion conversion, the prion "alleles" of course do not follow the Mendelian rule of equal segregation. However, deviation of this rule is a typical characteristic of all cytoplasmic (i.e. non-Mendelian) genes (see [5]).

In a striking parallel to DNA gene mutations, prionization can result in formation of multiple "protein alleles" — so-called "prion strains". Prion strains were initially described in mammals as infectious agents with different incubation periods [49]. They were long considered as the major obstacle to the "protein only" model of prion phenomena. However, it has recently been shown that "strain-specific" prion phenotypes correlate with the "strain-specific" patterns of PrP^{Sc} proteolytic digestion, which could be reproduced during in vitro propagation of PrP aggregates [50]. The yeast prion [PSI] can also exist in multiple states ("strains"), which differ from each other by both the intensity of translational readthrough and the frequency of mitotic loss [40]. Each "strain" of [PSI] is capable of reproducing its strain-specific characteristics in cell generations. While the molecular basis of the "strain" phenomenon is yet unknown, it is likely to involve either type of conformational alteration of the prion-forming protein, or certain properties of the prion aggregates, or both.

If prionization is analogous to the "forward mutation" in traditional terminology, "loss of prion" (i.e. conversion from prion isoform back to the "cellular", or "wild-type" isoform) would be analogous to "reverse mutation". Mitotic loss of the prion state in yeast can sometimes occur spontaneously, usually with low frequency. However, the "weak strains" of the [PSI] prion loose [PSI] relatively frequently, sometimes up to 1% per generation [40]. Even higher frequency of the spontaneous [PSI] loss was detected in the heterologous derivatives of [PSI], composed of the Sup35 protein with a prion-forming domain of the distant evolutionary origin [51–53] (see below). Certain treatments can increase [PSI] or [URE3] loss dramatically. Agents increasing loss of [PSI] or [URE3] (that is, "curing" yeast cells of a prion) could be considered as analogs of mutagens, that increase the rate of DNA mutations.

5. Protein mutagens

Some valuable information has been accumulated about chemical and other treatments that induce "reverse mutations" from prion to non-prion ("wild-type") state. Examples of "protein mutagens" that induce loss of [PSI] include both "conventional" (DNA) mutagens (such as UV and radiation) and agents potentially affecting protein folding (heat shock, osmotic stress, dimethylsulfoxide, ethanol, etc.) (see [25] for review) or assembly of the multiprotein cytoskeletal structures (latrunculin A) [54] (see Table 2). It is logical that while "DNA mutagens" affect nucleotide sequence of DNA, the "protein mutagens" should affect protein conformation and assembly — a "coding element" of the protein based inheritance. It is less clear why should conventional (DNA) mutagens affect protein based genetic elements as well. We will discuss one possible explanation for such a phenomenon later.

Probably the most efficient and most extensively investigated "cure" of [PSI] is guanidine-HCl (GuHCl) [25,55]. GuHCl also cures yeast cells of [URE3] [21]. GuHCl is a protein denaturing agent; however, the concentrations used in "prion-curing" experiments (1–5 mM) are too low for significant protein denaturation. Recent data [56] confirm that GuHCl effect on [PSI] is strictly generation dependent, suggesting that GuHCl is blocking proliferation of [PSI] rather than converting pre-existing [PSI] back into the "wild-type" form. GuHCl can possibly be compared to mutagens which block replication and cause deletions of genes or loss of whole DNA replicons. Whether GuHCl achieves this by affecting the prion protein directly or by inactivating the auxillary "helpers" (see below), remains to be understood.

Most conventional mutagens influence both "forward" and "reverse" DNA mutations. The agents, which cause loss of the prion ("reverse protein mutation") in most cases were not characterized in regard to their effect on prionization ("forward protein mutation"). In general, there is yet little known

Table 2
Protein mutagens

"Mutagenic" factor	Effect on prion		Effect on DNA genome		Molecular process affected
	[PSI]	[URE3]	Nuclear	Mitochondrial	
Environmental conditions					
Low temperature	Induction	Induction	None	None	Structure assembly
Heat or osmotic shock	Loss	?	None	Loss	Protein folding and stress response
Deep stationary phase	Induction	?	None	Alteration or loss	Stress response
DNA damaging agents (UV, radiation)	Loss	?	Alteration	Alteration or loss	DNA structure and stress response
Chemical agents					
Guanidine-HCl	Loss	Loss	None	Loss	Protein folding
DMSO	Loss	?	None	?	Protein folding and structure assembly
Ethanol, methanol	Loss	?	None	Loss	Protein folding and stress response
Latrunculin A	Loss	?	None	?	Actin cytoskeleton assembly

about the treatments or agents inducing prionization (see Table 2). It appears that prolonged incubation in the refrigerator (at about 4°C) increases appearance of the yeast prions ([57]; M. Aigle, personal communication, cited and confirmed in [58]; Y. Chernoff, A. Galkin and A. Borchsenius, unpublished data). Growth at low temperature (20–25°C) increases [PSI] induction by overproduced Sup35 protein of various origins [51,59]. Prolonged incubation in the stationary phase also appears to facilitate [PSI] formation in certain strains (Y. Chernoff et al., unpublished data). In general, the environmental factors and agents affecting prionization are yet to be characterized.

6. Genetic control of prion formation and propagation in yeast: protein mutators and antimutators

Our understanding of the molecular mechanisms of DNA mutagenesis has been driven by studying the mutants that increase (mutators) or decrease (antimutators) rates of mutations in the other genes. DNA mutators and antimutators have helped to elucidate the principles of DNA replication and repair, and have uncovered the role that replication and repair systems play in mutagenesis (for recent reviews, see [60–62]).

A similar approach can be applied to studying "protein mutagenesis". In yeast, the proteins affecting prion maintenance in *trans* were first identified by studying the effects of transient protein overproduction, the same tool that helped to identify Sup35 and Ure2 as prion carriers. Conventional genetic procedures allowing for inactivation of the corresponding genes were used on further stages.

6.1. Hsp104 role in [PSI] maintenance

The first protein shown to affect [PSI] maintenance in *trans* was the chaperone protein Hsp104. Increase in Hsp104 levels inhibits nonsense-suppression by [PSI] [58,63]. Moreover, yeast cells can be cured of [PSI] as a result of transient *Hsp104* overproduction [58]. Surprisingly, deletion of the *HSP104* gene also results in loss of the [PSI] state [58]. An "anti-PSI" effect of the *HSP104* deletion was recessive, while mutations inactivating the ATP-binding domains of Hsp104 protein exhibited a dominant negative effect on [PSI]. Apparently, the mutant Hsp104 protein is somehow outcompeting the wild-type Hsp104 protein and preventing it from performing its normal function on [PSI]. We hypothesized [58] that this could be due to a multimeric (hexameric) organization of the Hsp104 protein [64]. It is possible that "mutant" monomers inactivate the whole hexameric unit. Experiments are currently underway aimed at testing this hypothesis.

Overall, it looks like the Hsp104 chaperone plays a unique role in [PSI] propagation. The correct balance between Hsp104 and the [PSI]-forming protein Sup35 is extremely important. If this balance is shifted, reproduction of the prion state is impaired (Fig. 4A).

The Hsp104 chaperone belongs to the evolutionary conserved ClpB/Hsp100 family, members of which participate in various cellular processes [65]. In yeast,

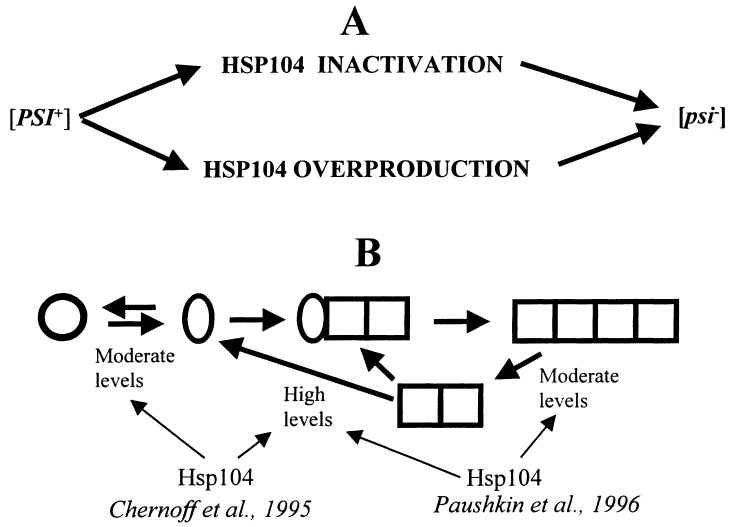


Fig. 4. Role of the yeast Hsp104 protein in [PSI] propagation. (A) Effects of Hsp104 inactivation and Hsp104 overproduction on [PSI] [58]. (B) Models explaining Hsp104 role in prion propagation [31,58]. The process of prion propagation is shown in accordance to the nucleated polymerization model (Fig. 2) but includes a partially unfolded intermediate state as confirmed by the recent data (for example, see [37]). According to Chernoff et al. [58], Hsp104 is required for the formation of this intermediate, which serves as a substrate for prion conversion. According to Paushkin et al. [31], Hsp104's role is to break prion polymers down into the oligomeric "seeds", initiating the next round of prion proliferation. Both models provide essentially the same explanation for the anti-prion effect of excess Hsp104. See more detailed comments in the text.

Hsp104 is responsible for so-called induced thermotolerance, that is, adaptation to severe heat shock induced by pre-incubation at mild heat shock conditions [66,67]. Hsp104 is also involved in the response to some other environmental stresses, as well as in the control of spore viability and long term viability of starving vegetative cells [67]. In molecular terms, Hsp104 is an ATPase [64], shown to promote solubilization of aggregated heat damaged proteins in vivo [68] and in vitro [69]. This "disaggregation" function of Hsp104 is achieved in cooperation with the other chaperones, Hsp70-Ssa [69,70] and Hsp40-Ydj1 [69].

Certain features of the Hsp104 effect on protein aggregates could be understood from a comparison with its *E. coli* homolog, ClpB. The bichaperone network, including the ClpB protein and DnaK–DnaJ–GrpE complex (a prokaryotic counterpart of the eukaryotic Hsp70–Hsp40), is responsible for resolubilization and refolding of misfolded proteins, previously aggregated in the absence of chaperones [71]. The experimental data suggest a model in which ClpB directly binds protein aggregates and, as a result of ATP hydrolysis, undergoes structural changes which increase the hydrophobic exposure of the aggregates. This allows the DnaK–DnaJ–GrpE complex to bind and promote disaggregation and refolding [71]. It is possible that Hsp104 protein and Hsp70–Hsp40 complex act on the yeast protein aggregates by a similar mechanism.

Initiation of protein "disaggregation" by Hsp104 could explain the fact that an excess of this protein interferes with propagation of prion aggregates. Indeed, increased proportion of soluble (wild-type) versus insoluble (prion) Sup35 protein has been detected in the [PSI$^+$] yeast cells overexpressing Hsp104 [30,31]. However, more difficult to explain is the observation that the absence of Hsp104 activity is so damaging for [PSI] reproduction. We initially hypothesized that Hsp104 activity could be involved in generation of the intermediate, a partially misfolded conformer of Sup35, that serves as a source for prion conversion [58]. For example, it is possible that alterations of the Hsp104 shift the balance between completely folded Sup35 protein and partly unstructured polypeptide which serves as a substrate for the process of aggregation (Fig. 4B). The role of unstructured polypeptide in the process of Sup35 aggregation has been supported

by in vitro studies [37]. Another model suggests that Hsp104 is needed for initiation of the "seeding" reaction: in the absence of Hsp104, prion aggregates can grow but cannot be broken down into the oligomeric "seeds" which initiate new rounds of prion reproduction. As a result, huge aggregates are eventually diluted and lost in cell divisions [31]. In either model, lack of Hsp104 activity blocks prion reproduction rather than removes prion conformers instantly (Fig. 4B). Therefore, Hsp104 inactivation should result in generation-dependent loss of prion. Our preliminary data support this prediction (Y. Chernoff, R. Wegrzyn and K. Bapat, unpublished data). However, [PSI] loss in the absence of Hsp104 appears to be more rapid than this would be expected if Hsp104 would simply block [PSI] proliferation without altering its structure. Together with the observation that the presence of Hsp104 influences two-hybrid interactions between Sup35N and some other proteins [72], this suggests a more complex role of Hsp104 in [PSI] maintenance.

Since Hsp104 levels are affected by certain environmental stresses, it was logical to suggest that some "protein mutagens" curing yeast cells of [PSI] do so by modifying levels or activity of Hsp104 [58]. Indeed, Hsp104 is induced by heat shock, ethanol and osmotic stress [67] — treatments known to cure yeast cells of [PSI] to a certain degree (see [25] for review). Moreover, Hsp104 is also expressed in response to UV or radiation induced DNA damage (T. Magee and K. McEntee, personal communication, quoted in [58]). Therefore, we have proposed that a "protein mutagenic" effect of UV is mediated by Hsp104 [58]. This removes a major obstacle to the "protein only" model of the [PSI] phenomenon: the observation that a "[PSI]-curing" effect of UV can be partially reversed by photoreactivation, that is, light-induced repair of DNA damage (see [73]). Indeed, photoreactivation decreases remaining DNA damage, that might in turn result in decreased Hsp104 induction. Thus, the effect of the "protein mutagens" could be mediated by chaperones in the same way as effects of DNA mutagens are mediated by DNA repair systems.

The most extensively characterized [PSI] curing agent, GuHCl, does also increase the levels of Hsp104 expression [58,74]. However, it is unlikely that the GuHCl effect on [PSI] is explained by Hsp104 induction. While the Sup35 solubilizing and [PSI] curing effect of overproduced Hsp104 could be relatively fast [30,75], the effect of GuHCl is generation dependent [56]. The Hsp104 inducing stress treatments such as ethanol stress or heat shock do not increase [PSI] curing effect of GuHCl [56]. Moreover, Hsp104 ATPase activity is inhibited in vitro by millimolar concentrations of GuHCl [69] – the same concentrations that are used to cure yeast cells of [PSI]. This means that even though Hsp104 levels are elevated in the presence of GuHCl, the Hsp104 activity is unlikely to be increased. In fact, it is possible that GuHCl could affect [PSI] propagation by inactivating (rather than inducing) Hsp104. However, our preliminary data indicate that kinetic parameters of [PSI] curing by GuHCl treatment and by Hsp104 inactivation are different from each other (Y. Chernoff, R. Wegrzyn and K. Bapat, unpublished data).

On the other hand, it is clear that [PSI] loss in response to environmental treatments cannot be solely explained by variations in Hsp104 levels or activity. For example, [PSI] is not eliminated at any detectable level by growth at increased but still permissive temperature ("mild heat shock", in contrast to severe heat shock) [76], by sporulation, or by incubation in the stationary phase (see [25] for review), despite the fact that all these conditions have been shown to induce Hsp104 [67]. This suggests that other proteins or physiological changes associated with these conditions could interfere with Hsp104 effects on [PSI]. Our further experiments in this direction were focused on the chaperones of the Hsp70 family, which are shown to interact with Hsp104 in disaggregating and refolding of heat damaged proteins (see above).

6.2. Effects of Hsp70 proteins on [PSI]

The major cytosolic subfamily of the Hsp70 family in yeast is Ssa, which includes four very closely related and highly homologous proteins, namely Ssa1, 2, 3, and 4 [77]. The total level of Ssa proteins in the cell is increased in response to high temperature, stationary phase and sporulation, although different members of the subfamily exhibit different modes of regulation. As discussed above, the Ssa1 protein (and possibly other members of the Ssa subfamily), together with Hsp104 and Ydj1, participate in disaggregating and refolding of heat damaged protein agglomerates [69]. Surprisingly, we have shown that overproduction of Ssa1 increases translational readthrough, caused by [PSI],

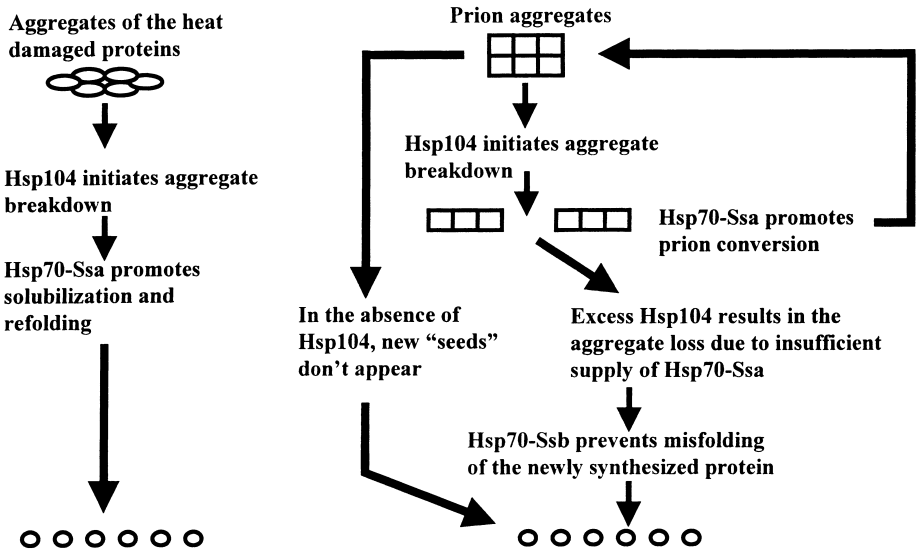


Fig. 5. Comparisons of the chaperone effects on heat-damaged aggregates and prion aggregates in yeast. Data on chaperone effects on temperature tolerance are from [68–71]. Data on chaperone effects on prions are from [58,59,78,79]. The model suggests that Hsp104 is responsible for the initial prion "seeding" (see Fig. 4B), while Hsp70-Ssa promotes prion conversion, and Hsp70-Ssb antagonizes it by preventing accumulation of misfolded protein serving as a conversion substrate. Alterations in the balance of Hsp104 and Hsp70 proteins result in prion loss. See more detailed comments in the text.

and prevents efficient [PSI] "curing" by overexpressed Hsp104 [78]. It appears that effects of Hsp70-Ssa on aggregates of heat damaged proteins and on prion aggregates diverged from each other. One possible explanation is that due to the high order structure of prion aggregates, Hsp70-Ssa protein recognizes them as legitimate subcellular structures rather than agglomerates of misfolded proteins. Therefore, Ssa1 helps to convert Sup35 protein back to the prion state, thus reversing an Hsp104 effect. In this way, Hsp70-Ssa actually promotes prion propagation in vivo. Such a model suggests that Hsp104 is responsible for the initial step of prion propagation in vivo (that is, generation of conversion intermediates or aggregation "seeds"), while Hsp70-Ssa works on the further step (that is, actual conversion of the intermediates into prions) (Fig. 5). Indeed, increased Ssa levels lead to increase of efficiency of nonsense-suppression in the [PSI$^+$] strains, apparently due to increased proliferation of the [PSI] prion [78]. The *ssa1* mutation has also been identified recently which causes a defect in [PSI] propagation [79]. Our preliminary data indicate that excess Ssa1 increases the efficiency of [PSI] induction by overproduced Sup35 (K. Allen and Y. Chernoff, unpublished data). Interestingly, the mammalian Hsp70 homologs have been shown to be involved in the protection of high order structures (e.g. cytoskeletal networks) during heat shock, suggesting that Hsp70 may assist in reassembly of the multiprotein complexes [80].

The "prion helper" effects of Hsp104 (at intermediate levels) and Hsp70-Ssa indicate that yeast prions have "learned" how to use cellular stress defense systems to their own advantage, in the same way as viruses and transposons employ the cellular DNA replication and repair machinery for their own reproduction. Since both Hsp104 and Hsp70-Ssa are induced during growth at high temperature, it is probable that Hsp70-Ssa is one of the factors protecting [PSI] from the curing effect of Hsp104 at these conditions.

Another yeast cytosolic Hsp70 subfamily, Ssb, includes two essentially identical proteins, namely Ssb1 and Ssb2. In contrast to the Ssa subfamily, the Ssb subfamily is constitutively expressed, is not induced in response to high temperature, and is not essential for viability [81]. Biochemical data suggest that Ssb proteins are associated with the translating ribosomes and nascent polypeptides [81,82]. Alterations of Ssb levels affect ubiquitin-dependent proteolysis [83,84],

leading to the suggestion that one of Ssb functions is the "proofreading" of protein folding, that is to say, identification of newly synthesized misfolded polypeptides and targeting them for degradation. We have shown that increased levels of Ssb enhance the "[PSI] curing" effect of excess Hsp104, while deletion of both SSB genes decreases the efficiency of [PSI] "curing" by excess Hsp104 and significantly increases the rate of spontaneous [PSI] formation [59]. It is possible that the Ssb protein normally antagonizes prion formation and reproduction by recognizing misfolded polypeptides, which could potentially be converted into prions, and targeting them for degradation (Fig. 5). Therefore, the double *ssb* deletion (*ssb1, 2Δ*) is the first example of a "protein mutator", increasing prionization (that is, "forward" protein mutation).

6.3. Universality and specificity of the chaperone effects on prions

Both Hsp104 and Hsp70 proteins belong to evolutionarily conserved groups of chaperones with a wide spectrum of action. This suggests that these chaperones could potentially affect prions other than [PSI]. Indeed, yeast Hsp104 has been shown to promote in vitro conversion of the mammalian PrP protein from the soluble to the insoluble (aggregated) isoform [85]. The Hsp104 inactivation also resulted in the loss of [URE3] in some strains [86], but not in all the strains (Y. Chernoff and S. Liebman, unpublished data, quoted in [42,44]). On the other hand, overproduction of Hsp104 failed to cure yeast cells of [URE3] ([86]; Y. Chernoff and S. Liebman, unpublished data, quoted in [42,44]). It has also been reported that "heterologous" [PSI] prions with the Sup35N domain of distant evolutionary origin are less sensitive to the overproduced Hsp104 than the endogenous *S. cerevisiae* [PSI] [52], although the curing effect of excess Hsp104 on "heterologous" [PSI] was still observed at higher Hsp104 concentrations (E. Lewitin and Y. Chernoff, unpublished data). The [PIN] element, a potential prion mentioned below, is also cured by Hsp104 inactivation but not by Hsp104 overproduction [94]. Effects of the Hsp70 proteins on prions other than [PSI] are yet to be tested. However, an excess of the Hsp40-Ydj1 protein, an Ssa cofactor, causes time-dependent loss of [URE3] [86], although it has no comparable effect on [PSI] (K. Allen and Y. Chernoff, unpublished data). Taken together, these data suggest that while Hsp104 indeed plays a role of the general regulator of prion propagation in the yeast cell, a certain specificity of prion-chaperone interactions can still be observed.

6.4. Other proteins and genetic elements, affecting prion formation and reproduction

In addition to the chaperones, we have identified another yeast protein affecting prion formation and reproduction. This is the cytoskeletal assembly protein Sla1. The known function of Sla1 is to assist in "nucleation" of microfilaments of actin cortical cytoskeleton [87], although Sla1 is neither essential for this process nor is proven to interact directly with actin. The two-hybrid screen has identified the C-terminal domain of Sla1 as a protein fragment interacting with the N-terminal (prion-forming) domain of Sup35 [72]. Quite remarkably, the two-hybrid interaction between Sup35N and Sla1C was inhibited by Hsp104 inactivation. Moreover, the mutant derivatives of the Sup35N bearing certain *PNM* ("[PSI] no more") mutations were unable to interact efficiently with Sla1C. In the [psi$^-$] strain lacking the Sla1 protein, de novo [PSI] formation induced by Sup35 (or Sup35N) overproduction, was significantly decreased, compared to the isogenic Sla1$^+$ strain [72]. Therefore, the *sla1Δ* manifests itself as an "antimutator" in regard to prionization. While the [PSI$^+$] strains lacking Sla1 protein were still able to maintain a prion, the "[PSI] curing" effect of agents such as dimethylsulfoxide or overproduced Hsp104 was significantly increased in such strains [72]. These results indicate that Sla1 protein plays an important, although not essential, role in prion formation and "recovery" from prion-curing treatments.

Molecular mechanisms of the Sla1 effect on [PSI] remain unclear. There is evidence of direct interactions between the actin cytoskeleton and components of the translational machinery, including elongation factor EF-1α [88–91], which is partially homologous to the C-terminal (release factor) domain of Sup35 [92,93]. Prolonged incubation of the yeast cells in the presence of latrunculin A, an agent disrupting the actin cytoskeleton, causes loss of [PSI] [54]. However, the fluorescence microscopy assays failed to detect a direct association between Sup35^{PSI+} aggregates and the cortical actin cytoskeleton [54]. On the other hand,

the Sup35N aggregates formed in vitro represent the amyloid-like fibers which resemble cytoskeletal networks [32,33]. It is possible that Sla1 protein is involved in assembly of the other intracellular fiber-like structures, in addition to the cortical actin cytoskeleton. In any case, the cytoskeletal assembly proteins play a role in assembly of multiprotein complexes, that is similar to the role played by chaperone proteins in assembly of the protein tertiary structures. This might explain why a cytoskeletal assembly helper is able to assist in prion assembly as well. This means that prionization could result from errors in the processes normally responsible for formation of the tertiary and quarternary protein structures.

Another genetic element, which affects prionization, is a non-Mendelian element [PIN] [94]. De novo [PSI] induction caused by overproduced Sup35 protein is efficient only in [PIN^+] strains [94]. Only some deletion derivatives of Sup35, bearing the Sup35N (prion-forming) domain with certain non-Sup35 extensions but lacking the Sup35C region, can induce [PSI] in the [pin^-] strains as well [57,94]. Neither translational suppression nor maintenance of the pre-existing [PSI] state are affected by [PIN] [57]. The [PIN] element is inherited in a non-Mendelian (cytoplasmic) fashion and "cured" by GuHCl treatment and by *hsp104* deletion (but not by Hsp104 overproduction) [94]. However, [PIN] maintenance does not require the Sup35N region, indicating that [PIN] is not controlled by the prion-forming domain of Sup35 protein [94]. It is an intriguing possibility that [PIN] could represent another prion, which is distinct from [PSI] but can "seed" [PSI] formation. In this case, we would have a protein-based genetic element that acts as a "protein mutator", increasing the rate of prionization of the other protein.

Efficiency of [PSI] induction by the overproduced Sup35 protein is also modified by variations in levels of another component of the yeast release factor complex, Sup45 (eRF1). Increased levels of the Sup45 protein antagonize the nonsense suppressor [95] and [PSI] inducing [96] effects of overproduced Sup35 protein. Apparently, interactions with Sup45 molecules, which physically bind Sup35 [97,98] and represent the normal partners of Sup35 in translation termination reaction [95,99], prevent self-aggregation and prion conversion of overproduced Sup35 protein. Interestingly, Sup45 levels do not appear to affect propagation and suppression efficiency of pre-existing [PSI] [96]. Moreover, it has been reported that at least in some [PSI^+] strains, the Sup45 molecules appear to be included in the $Sup35^{PSI+}$ aggregates and sequestered from the translational machinery [97].

In the case of [URE3], initial prion formation is also influenced by the protein which functionally interacts with Ure2, that is, Mks1 [100]. The Mks1 protein is a negative posttranscriptional regulator of Ure2 activity [101]. It is likely that Mks1 physically interacts with Ure2, although direct proof of such an interaction is missing thus far. The de novo formation of [URE3] is blocked, while propagation of pre-existing [URE3] is not affected in the strains lacking Mks1. Similar to the [PIN] element and Sup45 protein described above, presence or absence of Mks1 activity does not affect any characteristics of the pre-existing prion. The Mks1 protein itself is regulated by the Ras-cAMP pathway. Quite remarkably, $Ras2^{Val19}$ mutation which increases cAMP production, also blocks [URE3] formation, apparently due to inactivation of Mks1 [100]. These results point to a complex cellular control of [URE3] formation.

Information on the cellular control of prion formation and propagation in yeast available to date is summarized in Fig. 6.

6.5. DNA and protein repair systems: an overview

DNA mutators and antimutators uncover two groups of genetic elements: (1) sequence-specific *cis*-acting elements which control mutagenesis in the local regions of genome (mutation "hot spots", etc.); (2) *trans*-acting genes coding for the proteins which control the processes of DNA replication and repair in general (see [60–62] for review). In a similar way, protein "mutators" and "antimutators" target two groups of proteins. Some proteins influencing initial prion formation ("forward protein mutation") are involved in normal physiological interactions with the "prionized" substrates. Such proteins, namely Sup45 in case of [PSI] and Mks1 in case of [URE3], are specific to the particular prionized substrates (Sup35 and Ure2, respectively). Quite remarkably, these "protein-specific" factors have no effect on propagation of the pre-existing prion (defects of which result in "reverse protein mutations"). Apparently, the initial steps of prion formation involve competition between

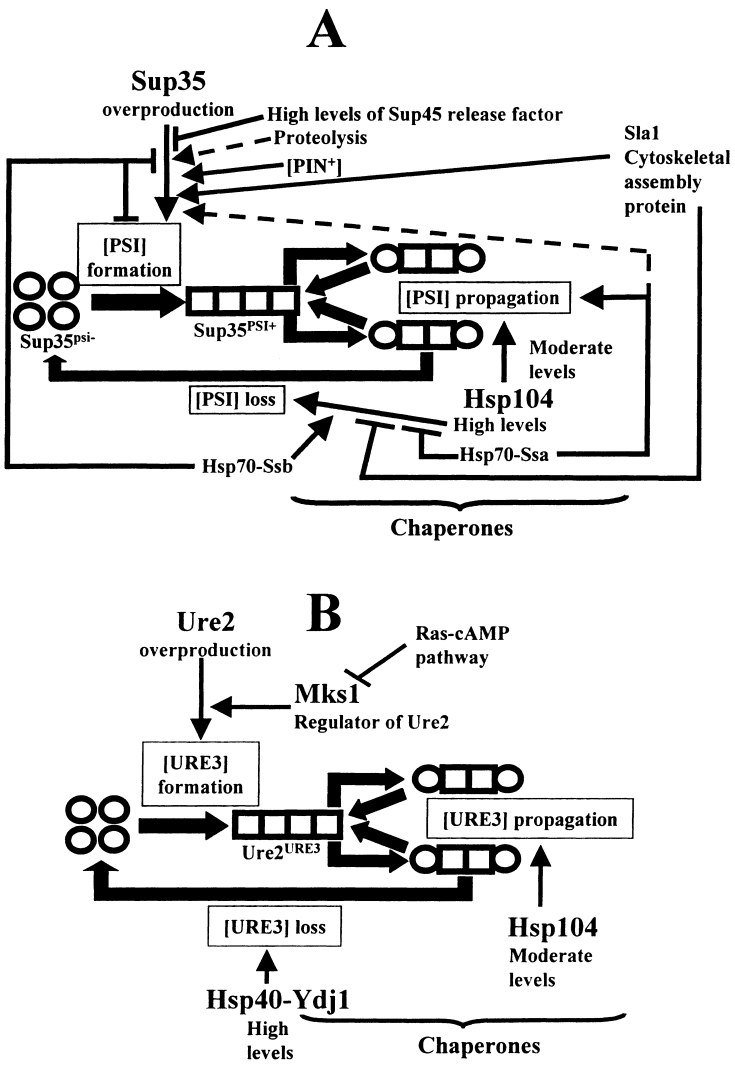


Fig. 6. Cellular control of prion propagation in yeast. (⟶) Positive effects, (⊣) negative effects. (A) Cellular control of [PSI] propagation. Data are from [40,58,59,72,78,94–96,138]. Effects based on preliminary data are indicated by discontinuous arrows. (B) Cellular control of [URE3] propagation. Data are from [86,100].

normal physiological partners and newly formed prion nuclei. Therefore, normal physiological partners occupy a position in protein mutagenesis system, that is equivalent to the *cis*-acting elements in DNA mutagenesis. In both cases, these factors reflect the normal location of the target within a certain region of DNA (DNA mutagenesis) or certain multiprotein complex (protein mutagenesis).

Once the stable prion is formed, the prion protein is removed from its normal physiological network, and its further behavior is controlled by *trans*-acting proteins of wide action spectra, specifically chaperones and cytoskeleton assembly helpers. Some of these proteins (e.g. Ssb and Sla1) appear to influence initial prion formation as well. At least some of the *trans*-acting proteins (e.g. Hsp104) effect prions of different structures and origins, suggesting existence of the general “prion replication” machinery operating in the yeast cell. An interesting parallel between the “DNA replication” and “prion replication” machiner-

Table 3
Comparison of the genetic elements affecting DNA and protein mutability

Genetic elements	DNA	Protein
Gene (or protein) specific		
Molecular basis	Adjacent pieces of DNA	Physiological partners of the prion protein
Role	Control of local DNA structure	Control of composition of the specific multiprotein complexes
Gene (or protein) non-specific		
Molecular basis	Components of DNA replication and repair machinery	Components of protein folding and assembly machinery
Role	Reproduction of DNA sequence	Reproduction of protein shape

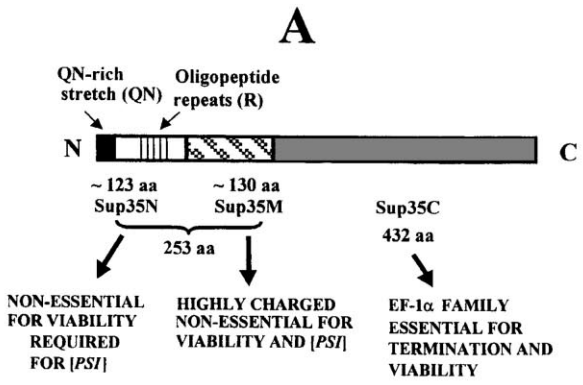
ies could be observed. The *trans*-acting components of the DNA replication/repair machinery control reproduction of DNA sequence, that is, a coding material for nucleic-based inheritance. In a similar way, the *trans*-acting components of the "prion replication" machinery control processes of protein folding and assembly of the multiprotein complexes, that is, generation and reproduction of the protein conformation, a "coding material" for "structural inheritance" (Table 3).

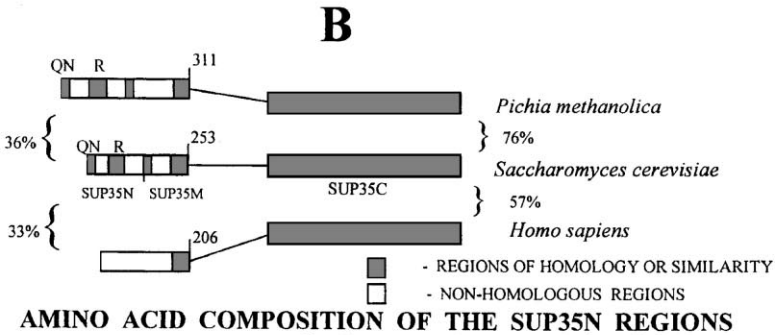
7. How widespread are protein-based genetic elements?

7.1. Evolutionary conservation of prion-forming potential

While Ure2 seems to be confined to fungi, Sup35 is an evolutionarily conserved protein detected in various groups of eukaryotes (see [102] for review). However, various regions of the Sup35 protein exhibit various modes of evolution. Deletion analysis [27,40,103] revealed that the *S. cerevisiae* Sup35 protein consists of three regions with distinct functions: (1) the C-proximal (Sup35C) region, which is essential for viability and translational termination and is partly homologous to translational elongation factor EF-1α; (2) the N-proximal (Sup35N) region, which is not essential for viability and termination, but is required for [PSI] induction and propagation; (3) the middle (Sup35M) region of unknown function, which is required for neither viability and termination nor [PSI] induction and propagation (see Fig. 7A). The Sup35N and Sup35C regions can perform their functions in *trans* (that is, when they are physically separated from each other) [27]. Moreover, fusion of the Sup35N region to an unrelated protein can lead to inactivation of such protein in [PSI$^+$] strains, apparently due to prion formation [104]. Evolutionary comparisons demonstrate that while the Sup35C region is greatly conserved from yeast to humans, the Sup35N and

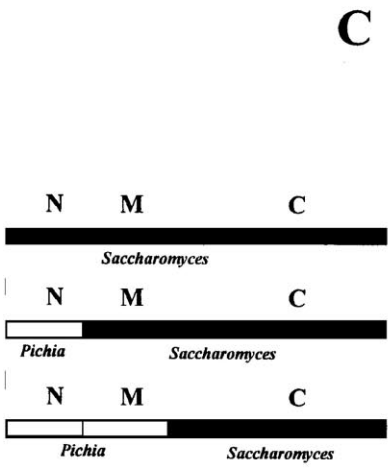
Fig. 7. Structure and evolution of the yeast Sup35 protein. (A) Structural organization of the *S. cerevisiae* Sup35 protein [92]; QN: the N-terminal Gln and Asn rich stretch [108], R: region of the oligopeptide repeats [73,109]. Since the exact boundary between the Sup35N and Sup35M regions is not known, it is assigned arbitrarily to the second Met residue at the position 124 of the Sup35 sequence. The minimal fragment of the Sup35 protein, shown to be able to maintain and induce [PSI] encompasses the first 113 amino acid residues [27,40,103]. (B) Evolution of the Sup35 (eRF3) protein from yeast to humans. Structural organizations of the Sup35 proteins of *S. cerevisiae* [92], a distantly related yeast *Pichia methanolica* [106], and *Homo sapiens* [105,107] are compared to each other. While the Sup35C regions are conserved in evolution. The Sup35N and Sup35M regions exhibit high level of divergence. However, the Gln/Asn rich stretch (QN) and the region of oligopeptide repeats (R) are conserved to a certain extent between *Saccharomyces* and *Pichia*. Certain patterns of amino acid composition of the Sup35N region appear to be conserved from yeast to humans. (C) Conservation of the prion-forming abilities of yeast Sup35 protein. Chimeric constructs bearing the Sup35N or Sup35NM portions of the *S. cerevisiae* Sup35 protein substituted by the corresponding regions of *P. methanolica* Sup35 protein, can be turned into a prion in *S. cerevisiae* by overproduction of the protein constructs including the *Pichia* Sup35N domain. On the other hand, the chimeric proteins with the *Pichia* Sup35N (or NM) domain cannot be turned into a prion by the pre-existing *Saccharomyces* prion, indicating existence of the "species barrier" in the yeast prion transmission. Overproduced prion forming domain of *Pichia* can induce the *Saccharomyces* Sup35 protein into a prion. However, such a heterologous induction is less efficient than the homologous induction. Data are from [51–53].





AMINO ACID COMPOSITION OF THE SUP35N REGIONS

	Gly, %	Gln, %	Asn, %
Saccharomyces cerevisiae	17.1	28.5	16.2
Pichia methanolica	11.2	18.7	26.1
Homo sapiens	22.2	2.6	5.1



Conversion into a prion by		
	pre-existing *Saccharomyces* [PSI⁺]	overproduced *Pichia* N or NM region
	+	+/-
	-	+
	-	+

Sup35M regions exhibit high degree of divergence even among various yeast genera [53,102,105–107] (see Fig. 7B). However, the Sup35N and Sup35M regions of various origins in most cases have more or less similar sizes and share highly unusual features of amino acid composition. For example, the Sup35N region is composed of mostly uncharged amino acids, while the Sup35M region is enriched by charged amino acids. Moreover, genetic analysis has identified two subregions of *S. cerevisiae* Sup35N which are involved in [PSI] formation and propagation: the Gln- and Asn-rich N-terminus [108] and the region of imperfect oligopeptide repeats [73,109]. A high concentration of the Asn residues is also characteristic for the N-proximal (prion-forming) domain of Ure2 [28], while oligopeptide repeats were found within the N-proximal half of mammalian PrP [10] (see above, Table 1). Both subregions can be found in at least some representatives of the other yeast genera, such as *Pichia*, *Candida*, *Kluyveromyces*, etc., despite the low level of overall conservation of the Sup35N amino acid sequences among these genera [53,106] (see also Fig. 7B).

The question arises whether the prion-forming potential of the Sup35N is conserved in evolution. Indeed, it has been shown that the chimeric Sup35 protein, with its Sup35N (or Sup35NM) region substituted by the corresponding piece of its *Pichia methanolica* or *Candida albicans* homolog, can form a prion in *S. cerevisiae* [51–53]. Prion formation was induced by overproduction of the homologous (i.e. *P. methanolica* or *C. albicans*) Sup35N (or Sup35NM) domain (see Fig. 7C), as shown previously for the endogenous *S. cerevisiae* Sup35 protein [40]. This strongly confirms evolutionary conservation of the prion-forming ability of the Sup35N domain. Interestingly, interspecies prion conversion (for example, from the Sup35 protein with *S. cerevisiae* Sup35NM to the Sup35 protein with *P. methanolica* Sup35NM) was inefficient [51–53], thus imitating the "species barrier" previously described for the mammalian PrP infection [110–112]. These results confirm that reproduction of the prion conformation requires a precise correspondence between the prion "seed" and target cellular protein. However, the species barrier could be partly overcome in some cases, when the heterologous protein was overproduced [51].

7.2. Fungal prions other than [PSI] and [URE3]

Conservation of the Sup35N prion-forming potential suggests that other proteins possessing the features of amino acid organization, which are similar to Sup35N and/or Ure2, could be able to form prions as well. Indeed, two proteins with Gln- and Asn-rich termini, New1 [53] and Rnq1 [113], were identified in *S. cerevisiae*. The N-proximal domain of New1 and the C-proximal domain of Rnq1 can promote aggregation and prion-like behavior when substituting for the Sup35N. While it is not yet known whether or not these proteins possess prion-forming ability on their own (that is, when they are not fused to Sup35), these results indicate that sequences with prion-forming potential are not rare in the *S. cerevisiae* genome. Indeed, the computer analysis identifies significant number of ORFs with poly-Gln runs in the *S. cerevisiae* genome [114]. Moreover, a number of these ORFs contain imperfect oligopeptide repeats as well (Y. Chernoff, unpublished data, quoted in [51]).

Another approach to look for new prions is based on the patterns of genetic behavior that are similar to those of [PSI] and [URE3]. This is how the fungal prion [Het^s] was recently identified. [Het^s] is a *Podospora* non-Mendelian element, which controls cytoplasmic incompatibility [115]. Similar to [PSI] and [URE3], [Het^s] requires the chromosomal gene for maintenance, is induced by overproduction of the corresponding cellular protein, and exhibits increased protease resistance. However, aggregation of the prion isoform of Het^s protein has yet to be proven.

The *S. cerevisiae* non-Mendelian element [PIN], described above, also possesses some features of the prion [94]. Quite remarkably, the only phenotypic expression of [PIN] known to date is to control initial formation of [PSI]. The [PIN] element is curable by guanidine-HCl [94], which also cures [PSI] and [URE3]. Similar to the yeast prions [PSI] and [URE3], [PIN] can appear de novo in the "cured" cells at low frequency, that appears to be increased during prolonged incubation at low temperature [57]. However, in contrast to [PSI], [PIN] is not sensitive to the *sup35NM* deletion, indicating that [PIN] does not depend on the Sup35 prion-forming domain [94]. While the molecular basis of [PIN] is not yet known, its prion-like nature seems likely, especially taking into consideration that [PIN], as does [PSI], requires

Hsp104 for its propagation [94]. If [PIN] is a prion, we might be dealing with a prion "cascade", where one prion influences the formation of another prion. Whether this occurs via a "cross-seeding" mechanism or through indirect regulatory effects, remains to be seen.

7.3. Amyloidoses and "neural inclusion" diseases

The very fact that several proteins of different structures, functions and origins possess prion-forming ability suggests that prion-like phenomena are widespread in nature. Indeed, so-called amyloidoses and other aggregation-related disorders in mammals (including humans) possess remarkable similarities to prion diseases. Aggregation-related disorders include such important diseases as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD), Amyotrophic Lateral Sclerosis (ALS), etc. [116–120]. Some of these diseases (e.g. HD and PD) are caused by "classic" DNA mutations in the corresponding genes, while others (e.g. AD and ALS) include both genetic and sporadic forms. In each case, development of the disease is associated with accumulation of abnormal aggregated proteins, deposited intracellularly or extracellularly in the form of "inclusion bodies" or amyloids. One type of DNA mutation leading to amyloid formation is expansion of homoaminoacid repeats, such as poly-Gln repeats in case of huntingtin, a causative agent of Huntington disease. Amyloids are generated by the "seeded polymerization" process, reminiscent of prion propagation. Sporadic amyloidoses and "neural inclusion" diseases are usually characterized by a "late onset", meaning that they appear late in life, when the protein folding and degradation system is apparently beginning to malfunction, and accumulation of the misfolded proteins occurs. Therefore, aggregation-related disorders are associated with cell aging. The major differences between prion diseases and other amyloidoses is the lack of infectivity in the latter case. However, infectivity of PrP remains a mystery and is probably related to unusually high protease resistance of the prion isoform of PrP. One should not expect that every protein with high aggregation potential would be capable of infection as well.

It is worth noting that the Hsp104 and Hsp70 proteins, which influence the yeast prion [PSI], as well as Hsp40 protein, previously shown to interact with Hsp70 and Hsp104 in thermotolerance [69], were also shown to interact with aggregates of poly-Gln expanded mammalian huntingtin introduced into the yeast cells [121,122]. In a similar way, it has been shown that yeast Hsp104, Hsp70 and Hsp40 chaperones counteract aggregation of poly-Gln containing proteins in *C. elegans* [123], and that Hsp70 and Hsp40 influence poly-Gln aggregation and/or aggregate toxicity in *Drosophila* [124–126]. Therefore, experiments on yeast prions can identify cellular proteins and conditions, which influence development of other aggregation-related disorders. As tests based on DNA-mutagenic activity in yeast and bacteria yielded the detection assays for potential carcinogenes and anti-carcinogenes [127], we believe that tests based on prion formation and propagation in yeast can produce the useful detection assays for identifying the agents promoting or preventing amyloidoses and neural inclusion disorders.

The examples discussed above indicate that a number of different proteins are capable of forming amyloid-like aggregates. Moreover, recent data suggest that most (if not all) proteins can form self-seeded amyloids in vitro under certain conditions [128,129]. If so, one should expect that a huge variety of cellular proteins would be able to undergo "prionization" (that is, "protein mutagenesis"). The actual question is not why can some proteins be turned into prion isoforms in vivo, but rather why does this normally occur with such a low frequency? Indeed, the efficiency of spontaneous [PSI] formation in [PIN^+] strains of *S. cerevisiae* is at the level of 10^{-6}, while in [pin^-] strains it is almost undetectable at the optimal growth conditions [59]. Apparently, the "folding proofreading" systems of the cell are generally capable of keeping prionization processes at low levels in the same way as "DNA proofreading" mechanisms antagonize spontaneous DNA mutability and protect the genome from destabilization.

7.4. Where to look for non-amyloid protein based genetic elements?

It should be noted that aggregating proteins are not the only proteins, which could potentially generate alternative self-reproducible isoforms. The self-modifying proteins, for example protein kinases, could in principle possess such an ability as well. Another

example of a system in which prion-like phenomena could play an important role is the formation of subcellular structures, such as cytoskeletal networks. Similar to prion aggregates, cytoskeletal formations are also generated and propagated by the "nucleated polymerization" process. It remains unknown whether structural organization of the cytoskeleton is completely determined by the information stored in DNA sequence, or directed in a certain way by the pre-existing cytoskeletal structure in a "template-like" mechanism. A parallel with "cortical inheritance" described above suggests that a template-like mechanism is rather more likely. Indeed, the cortical structure actually represents a complex external cytoskeletal formation. If structural features of such a formation are inherited in a template-like fashion, it is logical to expect that inheritance of the intracellular cytoskeletal structures would follow the same rules. Therefore, one might expect that new prions could be found among cytoskeletal proteins. The overlap between the proteins controlling prion formation and propagation, and the cytoskeletal assembly proteins, as demonstrated in case of the yeast Sla1 protein described above [72], makes such a possibility even more likely.

8. Could prions be adaptive?

PrP-dependent transmissible encephalopathies, as well as other amyloidoses and nuclear inclusion disorders, are dangerous diseases, that are usually fatal and incurable. In contrast, [PSI] and [URE3] do not kill yeast cells. However, the presence of [URE3] decreases growth rates [44]. The situation with [PSI] is less clear. One would expect [PSI] to be deleterious due to termination defect. However, no effect of [PSI] on the exponential growth rates was detected [25,44]. One explanation could be that natural terminators, protected by the nucleotide context [130], are probably less sensitive to shortage of the Sup35 function, compared to the artificially induced nonsense mutations used to monitor [PSI]. This needs further investigation, since the effect of [PSI] on the natural yeast terminators has never been systematically studied.

Our observations suggest that in some yeast strains, the presence of [PSI] significantly reduces viability in the deep stationary phase. Moreover, [PSI] dependent death of deep stationary cells is apparently oxidation-dependent, since overproduction of the antioxidant proteins superoxide dismutase and catalase could partly counteract the toxic effect of [PSI] (Y. Chernoff, J. Kumar, and G. Newnam, unpublished data). This effect could be compared to mammalian prion diseases and other amyloidoses, which usually affect terminally differentiated (that is, non-proliferating) cells and, in some cases, cause cell death in an oxidation-dependent manner. However, [PSI] toxicity in the stationary phase appears to be strain-specific. Further investigations are required to uncover the mechanism of this phenomenon.

Another situation in which [PSI] becomes deleterious is observed if Sup35 protein is overproduced even at moderate levels, such as 5–10-fold. This leads to severe inhibition of growth of the [PSI^+] cells [131–133]. Very high levels of the Sup35 protein or its fragments containing the prion-forming domain can also inhibit growth of [psi^-] strains [40]. Quite remarkably, toxicity of Sup35 fragments correlates with their ability to induce [PSI] and with the presence of genotypic elements required for [PSI] formation and propagation, in particular, [PIN^+] and functional Hsp104 [40,94]. This suggests that toxicity of hyper-overproduced Sup35 is mediated by efficient [PSI] induction and further inhibition of growth of the newly induced [PSI^+] cells.

Evidence for the possible adaptive role of [PSI] is less certain. In some genotypic backgrounds, [PSI^+] exponential cells were reported to exhibit increased thermotolerance, compared to isogenic [psi^-] derivatives obtained by guanidine-HCl treatment [134]. This effect could be indirect, via induction of the heat shock response prior to temperature treatment due to accumulation of abnormal mistranslated proteins [135]. It should be noted that the [PSI] effect on thermotolerance appears to be strain specific: in some genotypic backgrounds, [PSI] decreases rather than increases thermotolerance ([136]; Y. Chernoff, unpublished data). It should also be kept in mind that [psi^-] strains, obtained from [PSI^+] by guanidine-HCl treatment, could loose some other non-Mendelian elements in addition to [PSI], as described above for [PIN] and [URE3]. Differences in spectra of such non-Mendelian elements might potentially affect some phenotypic characteristics. Therefore, it could be premature to attribute the thermotolerance variations to [PSI] alone with certainty.

Another "adaptive" phenotype, associated with the presence of [PSI], is slightly increased resistance to the toxic effect of guanidine-HCl. This can be seen in *ssb1, 2Δ* strains which are hypersensitive to guanidine-HCl [59]. A systematic analysis of [PSI] effects on growth in various conditions, as well as on sensitivity to various stresses and toxic agents, was performed recently in S. Lindquist's laboratory [136]. Results suggest that the effects of [PSI] are greatly dependent on genotypic background. In certain conditions, the presence of [PSI] appeared to be "adaptive" in some strains and "inadaptive" in the others [136]. One explanation for such a discrepancy could be that yeast strains used in such experiments contain different spectra of the "silent" genes, interrupted by nonsense-mutations. Therefore, some of the effects attributed to [PSI] could be due to activation of such genes in result of [PSI] mediated translational read through. If such an ability of [PSI] to activate the naturally silent genes is proven, it would provide an important mechanism by which [PSI] contributes to the fitness of yeast cells.

It remains to be seen to what extent this phenomenon reflects a situation occurring in the natural conditions. Studying of the "adaptive" and "inadaptive" manifestations of [PSI] is greatly complicated by the fact that these assays are usually performed in laboratory strains bearing long chains of the genetic markers. The ancestors of these strains went through repeated cycles of mutagenesis. As a result, the laboratory strains may contain some unknown [PSI] suppressible nonsense mutations, in addition to known mutations. Different laboratory strains may have different combinations of the [PSI] suppressible nonsense mutations, that may explain the strain-specificity observed. The search for the presence of aggregated (prion) form of Sup35 protein in various natural and industrial isolates of yeast, which do not contain artificially introduced nonsense mutations, yielded no positive results thus far [51]. This outcome indicates that [PSI] could be rare in natural yeast populations, an observation that would rather be consistent with the general "inadaptivity" of [PSI].

On the other hand, the structural elements of the Sup35N region, which are responsible for [PSI] formation and propagation, appear to be conserved among the various yeast genera [53,106,109] (see also above, Fig. 7B). The actual ability of the Sup35N region to form a prion is also conserved in evolution as described above [51–53] (see also Fig. 7C). A comparative analysis of the polymorphism of Sup35NM region among various isolates of *S. cerevisiae* and *S. paradoxus* strongly suggests that the amino acid sequence of this region remains under selective pressure [137], despite the fact that the Sup35NM region is dispensable for viability. Since prion formation is the only biological function of the Sup35N domain known to date, such an analysis indicates that either prion formation itself could be adaptive under certain circumstances, or that prion formation requires the same structural elements of the Sup35 protein that are involved in the other (yet unknown) adaptive biological processes.

The first example of a prion clearly playing an important biological function is a *Podospora* prion [Het^{s}] [115]. Indeed, the [Het^{s}] prion is involved in the control of cytoplasmic incompatibility in *Podospora*. However, the prion fulfills its role by killing the cells of the strain which does not contain a prion. In this case, the "adaptive" role of the prion for one partner is achieved by killing another partner. In this context, this would be interesting to consider the possible relationship between prions and other amyloidoses, on the one hand, and the process of cell aging and death, on the other hand. As mentioned above, amyloidoses are usually characterized by a "late onset". In other words, this means that disease develops primarily in aging cells.

"Inadaptivity" of most prions and amyloid-like aggregates known to date raises a question: why is this mechanism so conserved in evolution, if evidence for its clear adaptive function is lacking? One could suggest that prions represent "selfish" genetic elements, which learned to evade the protein turnover processes and use cellular stress defense systems to their own advantage. Such an explanation is not without merit. In several cases (PrP, Ure2, Sup35), prion isoforms are more protease-resistant than soluble cellular isoforms. This could protect the prion from degradation by the cellular proteolytic systems. Moreover, short fragments of Sup35 [40,138] and Ure2 [28], which encompass the prion-forming domain, are more efficient in prion induction, compared to the complete protein. This suggests that prionization could be initiated by the proteolytic fragments seeking to evade further degradation. Such an extension of the "selfish DNA" model to the protein world might have interesting and

far-reaching evolutionary implications. Protein-based control elements like [Het^s] would then relate to the "selfish" prions in the same manner as regulatory DNA elements based on site-specific recombination (e.g. "phase switch" element in Salmonella [139]) relate to DNA based "selfish" phages and transposons.

However, another dimension of the problem should be mentioned as well. In considering prions as protein "mutants", one should not expect all (or even most) of these mutants to be adaptive. Most DNA mutations altering normal function of a gene lead to deleterious effects. However, a small percentage of mutations are neutral or beneficial and in such a way, they provide material for evolution. It is quite logical to expect that "protein mutations" would behave in the same way. If this is true, we might expect new "adaptive" prions to be found in the future. Moreover, the protein based hereditary elements could in general be less protected from the environmental changes, compared to the DNA based genomes. As indicated above, most "protein mutagenes" do not seem to affect DNA (see Table 2). At the same time, some "protein mutagens" (e.g. low or high temperature treatments) represent conditions which organisms are very likely to frequently encounter in natural environments. Changes in gene expression may also lead to very efficient induction or loss of prions, as shown in the experiments with overproduced proteins discussed above. If rapid restructuring of the population composition in response to environmental changes is needed, protein based genetic variation may provide an even more flexible and efficient tool for this than DNA based genetic variation. In general, the major function of protein based genetic changes would then be equivalent to that of DNA based genetic changes: to generate an inherited variability that produces material for new rounds of evolution.

Assignment of the genetic role to protein molecules removes the distinction between the genetic and enzymatic components of the cell, which seemed crystal clear two decades ago. In this respect, the prion concept completes work that had begun with discovery of RNA enzymatic functions [140]. One could ask a question: were not we too fast in rejecting the Lamarckian concept of inheritance of acquired traits? In classical genetics, it seemed evident that acquired phenotypic traits should not be inherited since genes (responsible for storage of genetic information) are distinct from enzymes (responsible for actual expression of the phenotypic traits). If this distinction is no longer valid, should not this mean that Jean-Baptiste Lamarck is back?

9. Is Lamarck back?

To his partial acquittal, Lamarck's sympathizers do usually cite "epigenic" effects, such as DNA methylation, etc. (see [141] as an example). However, it has been noted that "cortical memory", described by Beisson and Sonneborn [6], provides a mechanism which is even more directly related to Lamarck's concept [142]. The prion model extends this mechanism even further, making it applicable to the wide range of protein-based structures and phenotypic traits.

To avenge Lamarck completely, this should still be proven that protein-based genetic variation could generate newly acquired inherited traits, which are adaptive and adequate to the inducing factor. This postulate represented a foundation stone of Lamarckian evolutionary theory and was jealously defended by Lamarck's most fervent followers, such as the notorious Russian biologist T. Lysenko (see [143]). Proof of an adequate inherited adaptive change in protein structure is missing thus far. However, no one can now exclude the possibility that such a proof can be obtained in the future. For example, chaperone proteins involved in the stress response are capable of both regulating prion maintenance [58,59,78] and undergoing oligomerization or polymerization in certain conditions [144–146]. If stress related chaperones are found to acquire prion-like "seeding" abilities by themselves in response to certain changes in environmental conditions, the Lamarckian direct adaptation mechanism could finally be confirmed. Therefore, Lamarck is not back — not yet. But watch for him coming.

One could argue that prions described to date represent relatively isolated and exceptional examples, that would unlikely influence the general picture of evolution. However, such a conclusion is premature. Discovery of prions required sophisticated genetic (in yeast) and biochemical (in mammals) tools that were not available until recently. The materials discussed above suggest that prion-like potential (that is, ability to form stable alternative structures reproduced in a

template-like fashion) is widespread in nature. It is possible that some "maternal" and "non-Mendelian" traits of unknown molecular basis, described previously in plants and animals, originate from prion-like switches. Neither is the ability to form alternative conformations restricted to proteins. Recent data suggest that one and the same RNA sequence is able to fold into two different ribozymes with distinct enzymatic activities [147]. Rather, all this evidence indicates that traditional evolutionary concepts, not taking a "non-DNA" inheritance into consideration, can easily find themselves incomplete and outdated in the near future. The process of rethinking has begun among evolutionary biologists (e.g. [148,149]), but it is moving slowly thus far.

The prion model also raises another important question: does genome sequencing really tell us the whole story about genetic composition of the organism? If we admit prions into the genetic family (calling them and other such phenomena "epigenetic" changes words, but not the content), we have also to admit that large scale genomic projects should eventually fail to uncover all the information about genetic makeup of the organism. The example of yeast strains having identical DNA genomes but remaining genetically different in the prion-controlled traits proves this statement. This also means that "cloning" techniques would not necessarily produce genetically identical multicellular organisms, since only the DNA genome but not protein-based cytoplasmic inherited elements are reproduced in the existing "cloning" protocols [150]. As the ratio of "protein based" versus "nucleic acid based" inherited traits remains unclear, it appears that geneticists of the post-genomic and post-cloning era will not run out of the things to study any time soon.

Acknowledgements

I am grateful to the members of my laboratory, in particular, K. Allen, K. Bapat, G. Newnam and R. Wegrzyn, for the critical reading of manuscript and helpful suggestions. I thank S. Lindquist and R. Wickner for sharing some data prior to publication. This work was supported in parts by grants from the National Institutes of Health (R01GM58763) and from the Amyotrophic Lateral Sclerosis Association.

References

[1] L. Dunn, A Short History of Genetics, McGraw-Hill, New York, 1965.
[2] A.H. Sturtevant, A History of Genetics, Harper & Row, New York, 1965.
[3] R. Olby, Origins of Mendelism, Schocken Books, New York, 1966.
[4] J.D. Watson, F.H.C. Crick, A structure of deoxyribose nucleic acid, Nature 171 (1953) 737–738.
[5] A.J.F. Griffith, J.H. Miller, D.T. Suzuki, R.C. Lewontin, W.M. Gelbart, An Introduction to Genetic Analysis, 7th Edition, Freeman, New York, 2000.
[6] J. Beisson, T.M. Sonneborn, Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*, Proc. Natl. Acad. Sci. U.S.A. 53 (1965) 275–282.
[7] S.F. Ng, J. Frankel, 180 Degrees rotation of ciliary rows and its morphogenetic implications in *Tetrahymena pyriformis*, Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 1115–1119.
[8] C. Hyver, H. LeGuyader, Cortical memory in *Paramecium*: a theoretical approach to the structural heredity, C. R. Acad. Sci. 318 (1995) 375–380.
[9] S.B. Prusiner, Novel proteinaceous infectious particles cause scrapie, Science 216 (1982) 136–144.
[10] S.B. Prusiner, Molecular biology and genetics of prion diseases, Phil. Trans. R. Soc. Lond. Ser. B: Biol. Sci. 343 (1994) 447–463.
[11] S.B. Prusiner, M.R. Scott, S.J. De Armond, F.E. Cohen, Prion protein biology, Cell 93 (1998) 337–348.
[12] T. Alper, W.A. Cramp, D.A. Haig, M.C. Clarke, Does the agent of scrapie replicate without nucleic acid? Nature 214 (1967) 764–766.
[13] J.S. Griffith, Self-replication and scrapie, Nature 215 (1967) 1043–1044.
[14] B. Chesebro, Human TSE disease — viral or protein only? Nature Med. 3 (1997) 491–492.
[15] F.E. Cohen, K.M. Pan, Z. Huang, M. Baldwin, R.J. Fletterick, S.B. Prusiner, Structural clues to prion replication, Science 264 (1994) 530–531.
[16] P.M. Harrison, P. Bamborough, V. Daggett, S.B. Prusiner, F.E. Cohen, The prion folding problem, Curr. Op. Struct. Biol. 7 (1997) 53–59.
[17] P.T. Lansbury, B. Caughey, The chemistry of scrapie reaction: the "ice 9" metaphore, Chem. Biol. 2 (1995) 1–5.
[18] S.B. Prusiner, M.P. McKinley, K.A. Bowman, D.C. Bolton, P.E. Bendheim, D.F. Groth, G.G. Glenner, Scrapie prions aggregate to form amyloid-like birefringent rods, Cell 35 (1983) 349–358.
[19] S.J. DeArmond, M.P. McKinley, R.A. Barry, M.B. Braunfeld, J.R. McColloch, S.B. Prusiner, Identification of prion amyloid filaments in scrapie-infected brain, Cell 41 (1985) 221–235.
[20] D.A. Kocisko, J.H. Come, S.A. Priola, B. Chesebro, G.J. Raymond, P.T. Lansbury, B. Caughey, Cell-free formation of protease-resistant prion protein, Nature 370 (1994) 471–474.
[21] R.B. Wickner, [URE3] as an altered Ure2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*, Science 264 (1994) 566–569.

[22] F. Lacroute, Non-Mendelian mutation allowing ureidosuccinic acid uptake in yeast, J. Bacteriol. 106 (1971) 519–522.
[23] M. Aigle, F. Lacroute, Genetical aspects of [*URE3*], a non-mitochondrial cytoplasmically inherited mutation in yeast, Mol. Gen. Genet. 136 (1975) 327–335.
[24] B.S. Cox, Ψ, a cytoplasmic suppressor of super-suppressor in yeast, Heredity 20 (1965) 505–521.
[25] B.S. Cox, M.F. Tuite, C.S. McLaughlin, The Ψ factor of yeast: a problem in inheritance, Yeast 4 (1988) 159–178.
[26] S.M. Doel, S.J. McCready, C.R. Nierras, B.S. Cox, 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 (1994) 659–670.
[27] M.D. Ter-Avanesyan, A.R. Dagkesamanskaya, V.V. Kushnirov, V.N. Smirnov, The *SUP35* omnipotent suppressor gene is involved in the maintenance of the non-Mendelian determinant [*psi*$^+$] in the yeast *Saccharomyces cerevisiae*, Genetics 137 (1994) 671–676.
[28] D.C. Masison, R.B. Wickner, Prion-inducing domain of yeast Ure2p and protease-resistance of Ure2p in prion-containing cells, Science 270 (1995) 93–95.
[29] H.K. Edskes, V.T. Gray, R.B. Wickner, The [URE3] prion is an aggregated form of Ure2p that can be cured by overexpression of Ure2p fragments, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 1498–1503.
[30] M.M. Patino, J.J. Liu, J.R. Glover, S. Lindquist, Support for the prion hypothesis for inheritance of a phenotypic trait in yeast, Science 273 (1996) 622–626.
[31] S.V. Paushkin, V.V. Kushnirov, V.N. Smirnov, M.D. Ter-Avanesyan, Propagation of the yeast prion-like [*psi*$^+$] determinant is mediated by oligomerization of the *SUP35*-encoded polypeptide chain release factor, EMBO J. 15 (1996) 3127–3134.
[32] J.R. Glover, A.S. Kowal, E.C. Schirmer, M.M. Patino, J.J. Liu, S. Lindquist, Self-seeded fibers formed by Sup35, the protein determinant of [*PSI*$^+$], a heritable prion-like factor of *Saccharomyces cerevisiae*, Cell 89 (1997) 811–819.
[33] C.Y. King, P. Tittmann, H. Gross, R. Gebert, M. Aebi, K. Wuthrich, Prion-inducing domain 2–114 of yeast Sup35 protein transforms in vitro into amyloid-like filaments, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 6618–6622.
[34] K.L. Taylor, N. Cheng, R.W. Williams, A.C. Steven, R.B. Wickner, Prion domain initiation of amyloid formation in vitro from native Ure2p, Science 283 (1999) 1339–1343.
[35] C. Thual, A.A. Komar, L. Bousset, E. Fernandez-Bellot, C. Cullin, Structural characterization of *Saccharomyces cerevisiae* prion-like protein Ure2, J. Biol. Chem. 274 (1999) 13666–13674.
[36] M. Schlumpberger, H. Wille, M.A. Baldwin, D.A. Butler, I. Herskowitz, S.B. Prusiner, The prion domain of yeast Ure2p induces autocatalytic formation of amyloid fibers by a recombinant fusion protein, Protein Sci. 9 (2000) 440–451.
[37] T.R. Serio, A.G. Cashikar, A.S. Kowal, G.J. Sawicki, J.J. Moslehi, L. Serpell, M.F. Arnsdorf, S.L. Lindquist, Nucleated conformational conversion and the replication of conformational information by a prion determinant, Science 289 (2000) 1317–1321.
[38] S.V. Paushkin, V.V. Kushnirov, V.N. Smirnov, M.D. Ter-Avanesyan, In vitro propagation of the prion-like state of yeast Sup35 protein, Science 277 (1999) 381–383.
[39] Y.O. Chernoff, I.L. Derkach, S.G. Inge-Vechtomov, Multicopy *SUP35* gene induces *de-novo* appearance of *psi*-like factors in the yeast *Saccharomyces cerevisiae*, Curr. Genet. 24 (1993) 268–270.
[40] I.L. Derkatch, Y.O. Chernoff, V.V. Kushnirov, S.G. Inge-Vechtomov, S.W. Liebman, Genesis and variability of [PSI] prion factors in *Saccharomyces cerevisiae*, Genetics 144 (1996) 1375–1386.
[41] H.E. Sparrer, A. Santoso, F.C. Szoka, J.S. Weissman, Evidence for the prion hypothesis: induction of the yeast [PSI$^+$] factor by in vitro-converted Sup35 protein, Science 289 (2000) 595–599.
[42] S.W. Liebman, I.L. Derkatch, The yeast [*PSI*$^+$] prion: making sense of nonsense, J. Biol. Chem. 274 (1999) 1181–1184.
[43] R.B. Wickner, H.K. Edskes, M.L. Maddelein, K.L. Taylor, H. Moriyama, Prions of yeast and fungi: proteins as genetic material, J. Biol. Chem. 274 (1999) 555–558.
[44] R.B. Wickner, Y.O. Chernoff, Prions of yeast and fungi: [URE3], [PSI] and [Het-s] discovered as heritable traits, in: S.B. Prusiner (Ed.), Prion Biology and Diseases, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999, pp. 229–272.
[45] T.R. Serio, S.L. Lindquist, Protein-only inheritance in yeast: something to get [PSI$^+$]-ched about, Trends Cell Biol. 10 (2000) 98–105.
[46] A. Viswanathan, H.J. You, P.W. Doetsch, Phenotypic change caused by transcriptional bypass of uracil in nondividing cells, Science 284 (1999) 159–162.
[47] A. Viswanathan, J. Liu, P.W. Doetsch, *E. coli* polymerase bypass of DNA base damage: mutagenesis at the level of transcription, in: A.C. Find (Ed.), Molecular Strategies in Biological Evolution, The New York Academy of Sciences, New York, 1999; Annals 870, 386–389.
[48] R. Holliday, The inheritance of epigenetic defects, Science 238 (1987) 163–170.
[49] A.G. Dickinson, V.M. Meikle, Host-genotype and agent effects in scrapie incubation: change in allelic interaction with different strains of agent, Mol. Gen. Genet. 112 (1971) 73–79.
[50] R.A. Bessen, D.A. Kocisko, G.J. Raymond, S. Nandan, P.T. Lansbury, B. Caughey, Non-genetic propagation of strain-specific properties of scrapie prion protein, Nature 375 (1995) 698–700.
[51] Y.O. Chernoff, A.P. Galkin, E. Lewitin, T.A. Chernova, G.P. Newnam, S.M. Belenkiy, Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein, Mol. Microbiol. 35 (2000) 865–876.
[52] V.V. Kushnirov, N.V. Kochneva-Pervukhova, M.B. Chechenova, N.S. Frolova, M.D. Ter-Avanesyan, Prion properties of the Sup35 protein of yeast *Pichia methanolica*, EMBO J. 19 (2000) 324–331.
[53] A. Santoso, P. Chien, L.Z. Osherovich, J.S. Weissman, Molecular basis of a yeast prion species barrier, Cell 100 (2000) 277–288.

[54] P.A. Bailleul-Winslett, G.P. Newnam, R.D. Wegrzyn, Y.O. Chernoff, An anti-prion effect of the anticytoskeletal drug latrunculin A in yeast, Gene Expression (2000), in press.
[55] M.F. Tuite, C.R. Mundy, B.S. Cox, Agents that cause a high frequency of genetic change from [*psi*$^+$] to [*psi*$^-$] in *Saccharomyces cerevisiae*, Genetics 98 (1981) 691–711.
[56] S.S. Eaglestone, L.W. Ruddock, B.S. Cox, M.F. Tuite, 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 (2000) 240–244.
[57] I.L. Derkatch, M.E. Bradley, S.V. Masse, S.P. Zadorsky, G.V. Polozkov, S.G. Inge-Vechtomov, S.W. Liebman, Dependence and independence of [*PSI*$^+$] and [*PIN*$^+$]: a two-prion system in yeast? EMBO J. 19 (2000) 1942–1952.
[58] Y.O. Chernoff, S.L. Lindquist, B. Ono, S.G. Inge-Vechtomov, S.W. Liebman, Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [*psi*$^+$], Science 268 (1995) 880–884.
[59] Y.O. Chernoff, G.P. Newnam, J. Kumar, K. Allen, A.D. Zink, Evidence for a protein mutator in yeast: role of the Hsp70-related chaperone Ssb in formation, stability, and toxicity of the [*PSI*] prion, Mol. Cell. Biol. 19 (1999) 8103–8112.
[60] P.M. Burgers, Eukaryotic DNA polymerases in DNA replication and DNA repair, Chromosoma 107 (1998) 218–227.
[61] J. Jiricny, Eukaryotic mismatch repair: an update, Mutat. Res. 409 (1998) 107–121.
[62] J.P. Horst, T.H. Wu, M.G. Marinus, *Escherichia coli* mutator genes, Trends Microbiol. 7 (1999) 29–36.
[63] Y.O. Chernoff, B. Ono, Dosage-dependent modifiers of *psi*-dependent omnipotent suppression in yeast, in: A.J.P. Brown, M.F. Tuite, J.E.G. McCarthy (Eds.), Protein Synthesis and Targeting in Yeast, NATO ASI Ser. H: Cell Biology, Vol. 71, Springer, Berlin, 1993, pp. 101–110.
[64] D.A. Parsell, Y. Sanchez, J.D. Stitzel, S. Lindquist, Hsp104 is a highly conserved protein with two essential nucleotide-binding sites, Nature 353 (1991) 270–273.
[65] E.C. Schirmer, J.R. Glover, M.A. Singer, S. Lindquist, HSP100/Clp proteins: a common mechanism explains diverse functions, Trends Biochem. 21 (1996) 289–296.
[66] Y. Sanchez, S.L. Lindquist, Hsp104 required for induced thermotolerance, Science 248 (1990) 1112–1115.
[67] Y. Sanchez, J. Taulien, K.A. Borkovich, S. Lindquist, Hsp104 is required for tolerance to many forms of stress, EMBO J. 11 (1992) 2357–2364.
[68] D.A. Parsell, A.S. Kowal, M.A. Singer, S. Lindquist, Protein disaggregation mediated by heat-shock protein Hsp104, Nature 372 (1994) 475–478.
[69] J.R. Glover, S. Lindquist, Hsp104, Hsp70 and Hsp40: a novel chaperone system that rescues previously aggregated proteins, Cell 94 (1998) 1–20.
[70] Y. Sanchez, D.A. Parsell, J. Taulien, J.L. Vogel, E.A. Craig, S. Lindquist, Genetic evidence for a functional relationship between Hsp104 and Hsp70, J. Bacteriol. 175 (1993) 6484–6491.
[71] P. Goloubinoff, A. Mogk, A.P. Zvi, T. Tomoyasu, B. Bukau, Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 13732–13737.
[72] P.A. Bailleul, G.P. Newnam, J.N. Steenbergen, Y.O. Chernoff, Genetic study of interactions between the cytoskeletal assembly protein sla1 and prion-forming domain of the release factor Sup35 (eRF3) in *Saccharomyces cerevisiae*, Genetics 153 (1999) 81–94.
[73] B.S. Cox, Prion-like factors in yeast, Curr. Biol. 4 (1994) 744–748.
[74] S. Lindquist, M.M. Patino, Y.O. Chernoff, A.S. Kowal, M.A. Singer, K.-H. Lee, T. Blake, S.W. Liebman, The role of Hsp104 in stress tolerance and [PSI$^+$] propagation in *Saccharomyces cerevisiae*, Cold Spring Harbor Symp. Quant. Biol. 60 (1995) 451–460.
[75] Y.O. Chernoff, S.W. Liebman, M.M. Patino, S.L. Lindquist, Response from Chernoff et al. [Re: R.B. Wickner, Prions of yeast and heat-shock protein 104: 'coprion' and cure], Trends Microbiol. 3 (1995) 369.
[76] A. Singh, C. Helms, F. Sherman, Mutation of the non-Mendelian suppressor, *psi*$^+$, in yeast by hypertonic media, Proc. Natl. Acad. Sci. U.S.A. 77 (1979) 1952–1956.
[77] M. Werner-Washburne, D.E. Stone, E.A. Craig, Complex interactions among members of an essential subfamily of *hsp70* genes in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 7 (1987) 2568–2577.
[78] G.P. Newnam, R.D. Wegrzyn, S.L. Lindquist, Y.O. Chernoff, Antagonistic interactions between yeast chaperones Hsp104 and Hsp70 in prion curing, Mol. Cell. Biol. 19 (1999) 1325–1333.
[79] G. Jung, G. Jones, R.D. Wegrzyn, D.C. Masison, A role for cytosolic Hsp70 in yeast [PSI$^+$] prion propagation and [PSI$^+$] as a cellular stress, Genetics 156 (2000) 559–570.
[80] P. Liang, T.H. McRae, Molecular chaperones and the cytoskeleton, J. Cell Sci. 110 (1997) 1431–1440.
[81] R.J. Nelson, T. Ziegelhoffer, C. Nicolet, M. Werner-Washburne, E.A. Craig, The translation machinery and 70 kDa heat shock protein cooperate in protein synthesis, Cell 71 (1992) 97–105.
[82] C. Pfund, N. Lopez-Hoyo, T. Ziegelhoffer, B.A. Schilike, P. Lopez-Buesa, W.A. Walter, M. Wiedmann, E.A. Craig, The molecular chaperone Ssb from *Saccharomyces cerevisiae* is a component off the ribosome-nascent chain complex, EMBO J. 17 (1998) 3981–3989.
[83] M. Ohba, A 70-kDa heat shock cognate protein suppresses the defects caused by a proteasome mutation in *Saccharomyces cerevisiae*, FEBS Lett. 351 (1994) 263–266.
[84] M. Ohba, Modulation of intracellular protein degradation by SSB1-SIS1 chaperon system in yeast *Saccharomyces cerevisiae*, FEBS Lett. 409 (1997) 307–311.
[85] S.K. DebBurman, G.J. Raymond, B. Caughey, S. Lindquist, Chaperone supervised conversion of prion protein to its protease-resistant form, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 13938–13943.
[86] H. Moriyama, H.K. Edskes, R.B. Wickner, [URE3] prion propagation in *Saccharomyces cerevisiae*: requirement for

chaperone Hsp104 and curing by overexpressed chaperone Ydj1p, Mol. Cell. Biol. 20 (2000) 8916–8922.
[87] D.A. Holtzman, S. Yang, D.G. Drubin, Synthetic-lethal interactions identify two novel genes, *SLA1* and *SLA2*, that control membrane cytoskeleton assembly in *Saccharomyces cerevisiae*, J. Cell. Biol. 122 (1993) 635–644.
[88] F. Yang, M. Dema, V. Warren, S. Dharmawardhane, J. Condulis, Identification of an actin-binding protein from *Dictyostelium* as elongation factor 1α, Nature 347 (1990) 494–496.
[89] B.T. Edmonds, ABP50: an actin-binding elongation factor 1α from *Dictyostelium discoideum*, J. Cell. Biochem. 52 (1993) 134–139.
[90] M. Suda, M. Fukui, Y. Sogabe, K. Sato, A. Morimatsu, R. Arai, F. Motegi, T. Miyakawa, I. Mabuchi, D. Hirata, Overproduction of elongation factor 1alpha, an essential translational component, causes aberrant cell morphology by affecting the control of growth polarity in fission yeast, Genes Cells 4 (1999) 517–527.
[91] O. Numata, Y. Kurasawa, K. Gonda, Y. Watanabe, Tetrahymena elongation factor-1 alpha is localized with calmodulin in the division furrow, J. Biochem. 127 (2000) 51–56.
[92] V.V. Kushnirov, M.D. Ter-Avanesyan, M.V. Telckov, A.P. Surguchov, V.N. Smirnov, S.G. Inge-Vechtomov, Nucleotide sequence of the *SUP2* (*SUP35*) gene of *Saccharomyces cerevisiae*, Gene 66 (1988) 45–54.
[93] P.G. Wilson, M.R. Culbertson, Suf12 suppressor protein of yeast: a fusion protein related to the EF-1α family of elongation factors, J. Mol. Biol. 199 (1988) 559–573.
[94] I.L. Derkatch, M. Bradley, P. Zhou, Y.O. Chernoff, S.W. Liebman, Genetic and environmental factors affecting the de novo appearance of the [PSI^+] prion in *Saccharomyces cerevisiae*, Genetics 147 (1997) 507–519.
[95] I. Stansfield, K.M. Jones, V.V. Kushnirov, A.R. Dagkesamanskaya, A.I. Poznyakovski, S.V. Paushkin, C.R. Nierras, B.S. Cox, M.D. Ter-Avanesyan, M.F. Tuite, The products of the SUP45 (eRF1) and *SUP35* genes interact to mediate translation termination in *Saccharomyces cerevisiae*, EMBO J. 14 (1995) 4365–4373.
[96] I.L. Derkatch, M. Bradley, S.W. Liebman, Overexpression of the *SUP45* gene encoding a Sup35p-binding protein inhibits the induction of the de novo appearance of the [PSI^+] prion, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 2400–2405.
[97] S.V. Paushkin, V.V. Kushnirov, V.N. Smirnov, M.D. Ter-Avanesyan, Interaction between yeast Sup45p (eRF1) and Sup35p (eRF3) polypeptide chain release factors: implications for prion-dependent regulation, Mol. Cell Biol. 17 (1997) 2798–2805.
[98] K. Ebihara, Y. Nakamura, C-terminal interaction of translational release factors eRF1 and eRF3 of fission yeast: G-domain uncoupled binding and the role of conserved amino acids, RNA 5 (1999) 739–750.
[99] G. Zhouravleva, L. Frolova, X. Le Goff, R. Le Guellec, S. Inge-Vechtomov, L. Kisselev, M. Philippe, Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3, EMBO J. 14 (1995) 4065–4072.
[100] H.K. Edskes, R.B. Wickner, A protein required for prion generation: [URE3] induction requires the Ras-regulated Mks1 protein, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 6625–6629.
[101] H.K. Edskes, J.A. Hanover, R.B. Wickner, Mks1p is a regulator of nitrogen catabolism upstream of Ure2p in *Saccharomyces cerevisiae*, Genetics 153 (1999) 585–594.
[102] Y.O. Chernoff, M.V. Ptyushkina, M.G. Samsonova, G.I. Sizonencko, Y.I. Pavlov, M.D. Ter-Avanesyan, S.G. Inge-Vechtomov, Conservative system for dosage-dependent modulation of translational fidelity in eukaryotes, Biochimie 74 (1992) 455–461.
[103] M.D. Ter-Avanesyan, V.V. Kushnirov, A.R. Dagkesamanskaya, S.A. Didichenko, Y.O. Chernoff, S.G. Inge-Vechtomov, V.N. Smirnov, Deletion analysis of the *SUP35* gene of the yeast *Saccharomyces cerevisiae* reveals two non-overlapping functional regions in the encoded protein, Mol. Microbiol. 7 (1993) 683–692.
[104] L. Li, S. Lindquist, Creating a protein-based element of inheritance, Science 287 (2000) 661–664.
[105] S. Hoshino, H. Miyazawa, T. Enomoto, F. Hanaoka, Y. Kikuchi, A. Kikuchi, M. Ui, A human homologue of the yeast *GST1* gene codes for a GTP-binding protein and is expressed in a proliferation-dependent manner in mammalian cells, EMBO J. 8 (1989) 3807–3814.
[106] V.V. Kushnirov, M.D. Ter-Avanesyan, S.A. Didichenko, V.N. Smirnov, Y.O. Chernoff, I.L. Derkach, O.N. Novikova, S.G. Inge-Vechtomov, M.A. Neistat, I.I. Tolstorukov, Divergence and conservation of *SUP2* (*SUP35*) gene of yeasts *Pichia pinus* and *Saccharomyces cerevisiae*, Yeast 6 (1990) 461–472.
[107] O. Jean-Jean, X. LeGoff, M. Philippe, Is there a human [*psi*]? C. R. Acad. Sci. Paris, Sciences de la vie/Life Sciences 319 (1996) 487–492.
[108] A.H. De Pace, A. Santoso, P. Hillner, J.S. Weissman, A critical role for amino-terminal glutamine/asparagine repeats in the formation and propagation of a yeast prion, Cell 93 (1998) 1241–1252.
[109] V.V. Kushnirov, M.D. Ter-Avanesyan, V.N. Smirnov, Structural and functional similarity of Sup35p and Ure2p yeast proteins and mammalian prions, Mol. Biol. (Russ.) 29 (1995) 427–430.
[110] J.C. Bartz, D.I. McKenzie, R.A. Bessen, R.F. Marsh, J.M. Aiken, Transmissible mink encephalopathy species barrier effect between ferret and mink: PrP gene and protein analysis, J. Gen. Virol. 75 (1994) 2947–2953.
[111] M. Bruce, A. Chree, I. McConnell, J. Foster, G. Pearson, H. Fraser, Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier, Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci. 343 (1994) 405–411.
[112] D.A. Kocisco, S.A. Priola, G.J. Raymond, B. Chesebro, P.T. Lansbury, B. Caughey, Species specificity in the cell free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 3923–3927.
[113] N. Sondheimer, S. Lindquist, Rnq1: an epigenetic modifier of protein function in yeast, Mol. Cell 5 (2000) 163–172.

[114] E.T. Young, J.S. Sloan, K. Van Riper, Trinucleotide repeats are clustered in regulatory genes in *Saccharomyces cerevisiae*, Genetics 154 (2000) 1053–1068.
[115] V. Coustou, C. Deleu, S. Saupe, J. Begueret, The protein product of the het-s heterokaryon incompatibility gene of the fungus *Podospora anserina* behaves as a prion analog, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 9773–9778.
[116] A. Kakizuka, Protein precipitation: a common etiology in neurodegenerative disorders? Trends Genet. 14 (1998) 396–402.
[117] E.H. Koo, P.T. Lansbury, J.W. Kelly, Amyloid diseases: abnormal protein aggregation in neurodegeneration, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 9989–9990.
[118] J.N. Buxbaum, C.E. Tagoe, The genetics of the amyloidoses, Ann. Rev. Med. 51 (2000) 543–569.
[119] R. Kruger, T. Muller, O. Riess, Involvement of alpha-synuclein in Parkinson's disease and other neurodegenerative disorders, J. Neural. Trans. 107 (2000) 31–40.
[120] H.Y. Zoghbi, H.T. Orr, Glutamine repeats and neurodegeneration, Ann. Rev. Neurosci. 23 (2000) 217–247.
[121] S. Krobitsch, S. Lindquist, Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 1589–1594.
[122] P.J. Muchowski, G. Schaffar, A. Sittler, E.E. Wanker, M.K. Hayer-Hartl, F.U. Hartl, Hsp70 and Hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 7841–7846.
[123] S.H. Satyal, E. Schmidt, K. Kitagawa, N. Sondheimer, S. Lindquist, J.M. Kramer, R.I. Morimoto, Polyglutamine aggregates alter protein folding homeostasis in Caenorhabditis elegans, Proc. Natl. Acad. Sci. U.S.A. 23 (2000) 5750–5755.
[124] Y. Chai, S.L. Koppenhafer, N.M. Bonini, H.L. Paulson, Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease, J. Neurosci. 19 (1999) 10338–10347.
[125] J.M. Warrick, H.Y. Chan, G.L. Gray-Board, Y. Chai, H.L. Paulson, N.M. Bonini, Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70, Nature Genet. 23 (1999) 425–428.
[126] P. Kazemi-Esfarjani, S. Benzer, Genetic suppression of polyglutamine toxicity in *Drosophila*, Science 287 (2000) 1837–1840.
[127] B.N. Ames, J. McCann, E. Yamasaki, Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res. 31 (1975) 347–363.
[128] F. Chiti, P. Webster, N. Taddei, A. Clark, M. Stefani, G. Ramponi, C.M. Dobson, Designing conditions for in vitro formation of amyloid protofilaments and fibrils, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 3590–3594.
[129] C.M. Dobson, Protein misfolding, evolution and disease, Trends Biochem. Sci. 24 (1999) 329–332.
[130] B. Bonetti, L. Fu, J. Moon, D.M. Bedwell, The efficiency of translation termination is determined by a synergistic interplay between upstream and downstream sequences in *Saccharomyces cerevisiae*, J. Mol. Biol. 251 (1995) 334–345.
[131] Y.O. Chernoff, I.L. Derkach, V.L. Tikhomirova, A.R. Dagkesamanskaya, M.D. Ter-Avanesyan, S.G. Inge-Vechtomov, Nonsense-suppression by amplification of translation protein factor gene, Doklady Akad. Nauk SSSR: Biol. Sci. (Russ.) 301 (1988) 1227–1229.
[132] Y.O. Chernoff, S.G. Inge-Vechtomov, I.L. Derkach, M.V. Ptyushkina, O.V. Tarunina, A.R. Dagkesamanskaya, M.D. Ter-Avanesyan, Dosage-dependent translational suppression in yeast *Saccharomyces cerevisiae*, Yeast 8 (1992) 489–499.
[133] A.R. Dagkesamanskaya, M.D. Ter-Avanesyan, Interactions of the yeast omnipotent suppressors *SUP1* (*SUP45*) and *SUP2* (*SUP35*) with non-Mendelian factors, Genetics 128 (1991) 513–520.
[134] S.S. Eaglestone, B.S. Cox, M.F. Tuite, Translation termination efficiency can be regulated in *Saccharomyces cerevisiae* by environmental stress through a prion-mediated mechanism, EMBO J. 18 (1999) 1974–1981.
[135] C.M. Grant, M. Firoozan, M.F. Tuite, Mistranslation induces the heat-shock response in the yeast *Saccharomyces cerevisiae*, Mol. Microbiol. 3 (1989) 215–220.
[136] H.L. True, S.L. Lindquist, A yeast prion provides a mechanism for genetic variation and phenotypic diversity, Nature 407 (2000) 477–483.
[137] M.A. Jensen, H. True, Y.O. Chernoff, S. Lindquist, Molecular population genetics and evolution of a prion-like protein in *Saccharomyces cerevisiae*, Genetics (2001), under revision.
[138] N.V. Kochneva-Pervukhova, A.I. Poznyakovski, V.N. Smirnov, M.D. Ter-Avanesyan, C-terminal truncation of the Sup35 protein increases the frequency of de novo generation of a prion-based [PSI$^+$] determinant in *Saccharomyces cerevisiae*, Curr. Genet. 34 (1998) 146–151.
[139] M. Silverman, J. Zieg, M. Hilmen, M. Simon, Phase variation in Salmonella: genetic analysis of a recombinational switch, Proc. Natl. Acad. Sci. U.S.A. 76 (1979) 391–395.
[140] T.R. Cech, B.L. Bass, Biological catalysis by RNA, Ann. Rev. Biochem. 55 (1986) 599–629.
[141] M. Balter, Was Lamarck just a little bit right? Science 288 (2000) 38.
[142] O.E. Landman, The inheritance of acquired characteristics, Ann. Rev. Genet. 25 (1991) 1–20.
[143] Z.A. Medvedev, Lysenko and Stalin: commemorating the 50th anniversary of the August 1948 LAAAS Conference and the 100th anniversary of T.D. Lysenko's birth, 29 September 1898, Mutat. Res. 462 (2000) 3–11.
[144] C. King, E. Eisenberg, L. Greene, Polymerization of 70-kDa heat shock protein by yeast DnaJ in ATP, J. Biol. Chem. 270 (1995) 22535–22540.
[145] C.E. Angelidis, I. Lazaridis, G.N. Pagoulatos, Aggregation of hsp70 and hsc70 in vivo is distinct and temperature-dependent and their chaperone function is directly related to non-aggregated forms, Eur. J. Biochem. 259 (1999) 505–512.
[146] M. Haslbeck, S. Walke, T. Stromer, M. Ehrnsperger, H.E. White, S. Chen, H.R. Saibil, J. Buchner, Hsp26: a temperature-regulated chaperone, EMBO J. 18 (1999) 6744–6751.

[147] E.A. Shultes, D.P. Bartel, One sequence, two ribozymes: implications for the emergence of new ribozyme folds, Science 289 (2000) 448–452.
[148] M.D. Golubovsky, Mobile genetics and forms of heritable changes in eukaryotes, Biopolym. Cells 11 (1995) 29–38.
[149] M.D. Golubovsky, R.N. Tchuraev, Dynamic inheritance and epigenes, Priroda 4 (1997) 16–25 (in Russian).
[150] I. Wilmut, A.E. Schnieke, J. McWhir, A.J. Kind, K.H. Campbell, Viable offspring derived from fetal and adult mammalian cells, Nature 385 (1997) 810–813.