Epigenetics in the Extreme: Prions and the Inheritance of Environmentally Acquired Traits

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Science **330**, 629 (2010);
DOI: 10.1126/science.1191081

ulation is that repeat RNAs are the targeting signal, but whether the RNA signal (either siRNAs or larger RNAs) is received depends on the chromatin state of the alleles, which has been shown to differ between *B-I, B′*, and the single-copy repeat alleles that do not undergo paramutation (*7*). Another hypothesis that is not mutually exclusive is that differential production of paramutation-associated RNAs occurs in developing embryos (*6*), where paramutation is established (*8*) and where RNAs have not yet been examined. This latter hypothesis is exciting given that cis- and trans-acting small RNAs regulate epigenetic changes during gametogenesis, fertilization, and early zygotic development in multiple species (*9*).

Although the above evidence supports a role for RNA, other factors, such as protein-DNA interactions, could also be involved. For example, interactions between proteins that bind to the *b1* tandem repeats might mediate communication between alleles. Data consistent with that idea are that a transgene overexpressing a protein that binds to the *b1* tandem repeats and forms multimers, inducing a *B′*-like state in *B-I* (*10*). Another possible model, frequently discussed, is that the alleles communicate through DNA pairing (*1*, *2*). Although there is no experimental evidence demonstrating a role for DNA pairing, there is no evidence eliminating it either. It is of course possible that RNA, DNA, and protein interactions are all required for paramutation.

Why are repeats required for paramutation? Tandem repeats create a characteristic sequence at their junctions relative to single-copy sequences; the *b1* tandem repeat junctions have distinct chromatin structures, which have been hypothesized to affect silencing (*7*), potentially through specific proteins or RNAs that associate with these sequences. It has also been suggested that RNAs synthesized from repeats, but not a single-copy sequence, trigger silencing (*6*). A model proposed to explain how centromeric tandem repeats maintain heterochromatin silencing (*11*) offers an hypothesis for how tandem repeats could generate a distinct pool of RNAs relative to nonrepeats. That model suggests a mechanism by which multiple cycles of amplification of RNAs from tandem repeats [as outlined in (*9*)] results in distinct populations of RNA that span the full repeat sequence, as compared to RNA amplification from dispersed copies or single-copy sequences that have reduced sequence complexity (*11*).

Once paramutation is established (*8*), it is maintained through mitotic and meiotic cell divisions. Although the nature of the heritable molecule(s) is unknown, it is unlikely to be *b1* tandem repeat siRNAs, as mitotic silencing is maintained when a mutation dramatically reduces these siRNAs in juvenile and adult tissues (*3*). Analyses of cytosine methylation and histone modifications in *B-I* and *B′* revealed more cytosine methylation within the *b1* tandem repeats in *B′* relative to *B-I* (*7*), whereas histones associated with the *b1* repeats in both alleles did not carry modifications characteristic of silent chromatin. Future studies on the paramutation properties of mutants impaired in DNA methylation and various histone modifications should shed light on the potential role for these marks in paramutation. The observations that RdDM in *Arabidopsis* is associated with cytosine methylation and heterochromatin histone modifications (*4*), yet paramutation does not occur between RdDM silenced alleles (see below), leads to the speculation that paramutation involves additional mechanisms, such as RNA or proteins that remain associated with the *b1* repeats during mitosis and meiosis.

It is puzzling that RNAi-mediated heterochromatin in *S. pombe* and RdDM-silenced genes in *Arabidopsis* do not undergo paramutation (*4*, *5*). For example, specific alleles of *b1* and *FWA* in *Arabidopsis* are both silent when cytosine residues of the respective tandem repeats are methylated and active when hypomethylated. In both systems, the tandem repeats required for silencing are transcribed and produce small RNAs regardless of whether the alleles are active or silent. The methylated, silenced *FWA* allele can initiate trans methylation of an unmethylated transgene, yet, unlike the maize paramutation system, the unmethylated allele segregates normally and is active and unchanged (*12*). It is unclear whether the "natural" active *FWA* allele is protected from silencing, or the transgene is hypersensitive to silencing, or both (*12*). Additionally, the mechanism that makes *B-I* in maize highly sensitized to silencing is also unknown, although several hypotheses have been proposed (*13*).

The relationship with other RNA silencing pathways suggests that paramutation, despite being rare, may underlie fundamental mechanisms for gene regulation (*2*). Speculations on potential roles and consequences include that paramutation provides an adaptive mechanism through the transfer of favorable expression states to progeny, that paramutation could be a mechanism for establishing functional homozygosity in polyploids, and that it might function in inbreeding depression and hybrid vigor or inheritance associated with complex human diseases (*13*).

Independent of paramutation's function or frequency, our understanding of its mechanisms should shed light on potentially novel mechanisms for transmitting epigenetic information across generations.

References

1. M. Stam, *Mol. Plant* **2**, 578 (2009).
2. C. M. Suter, D. I. K. Martin, *Trends Genet.* **26**, 9 (2010).
3. M. A. Arteaga-Vazquez, V. L. Chandler, *Curr. Opin. Genet. Dev.* **20**, 156 (2010).
4. M. Matzke, T. Kanno, L. Daxinger, B. Huettel, A. J. M. Matzke, *Curr. Opin. Cell Biol.* **21**, 367 (2009).
5. S. I. S. Grewal, *Curr. Opin. Genet. Dev.* **20**, 134 (2010).
6. M. Arteaga-Vazquez *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 12986 (2010).
7. M. Haring *et al.*, *Plant J.* **63**, 366 (2010).
8. E. H. Coe Jr., *Genetics* **53**, 1035 (1966).
9. D. Bourc'his, O. Voinnet, *Science* **330**, 617 (2010).
10. K. Brzeska, J. Brzeski, J. Smith, V. L. Chandler, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 5516 (2010).
11. R. A. Martienssen, *Nat. Genet.* **35**, 213 (2003).
12. I. R. Henderson, S. E. Jacobsen, *Nature* **447**, 418 (2007).
13. V. L. Chandler, *Cell* **128**, 641 (2007).

10.1126/science.1191044

PERSPECTIVE

Epigenetics in the Extreme: Prions and the Inheritance of Environmentally Acquired Traits

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Prions are an unusual form of epigenetics: Their stable inheritance and complex phenotypes come about through protein folding rather than nucleic acid–associated changes. With intimate ties to protein homeostasis and a remarkable sensitivity to stress, prions are a robust mechanism that links environmental extremes with the acquisition and inheritance of new traits.

In its modern usage, "epigenetics" encompasses all mechanisms for the inheritance of biological traits that do not involve alterations of the coding sequence of DNA (*1*). Considered elsewhere in this issue are well-known epigenetic mechanisms that control access to DNA by modifying nucleotides or associated histones, or involve the transmission of information through RNA. Here, we discuss an extreme case of epigenetic inheritance with a mechanism that is not based on heritable changes in nucleic acid. Instead, it is based on robust self-propagating changes in the folding of certain proteins known as prions.

Prions operate outside the canonical steps of molecular biology's central dogma. As protein-

based elements of inheritance, prions perpetuate not by changing the way that genetic information is transcribed or translated but rather by co-opting the final step in the decoding of genetic information—protein folding. A key feature of prion-forming proteins is their ability to exist in very different stable conformational states. In addition to a "native" nonprion conformation, they occasionally fold into a prion conformation that then replicates itself by templating the conformational conversion of other molecules of the same protein. These changes in conformation profoundly alter the functions of the proteins involved, resulting in phenotypes specific to each determinant protein.

The idea that proteins could transmit information in a manner analogous to nucleic acids was first conceived to explain baffling infectious neurodegenerative diseases (such as Kuru and mad cow disease) (*2*). As evidence accumulated that these diseases did not require nucleic acids for transmission, the infectious agent was postulated to be a self-replicating protein. It is now clear that the prion does not synthesize itself from individual amino acids. Rather, it is a host-encoded protein in a conformation that is profoundly different from normal. The prion "replicates" simply by templating that conformation to other molecules of the protein. The initially mysterious and controversial nature of infectious prions created a stir that even today sometimes overshadows what we believe is a far more interesting aspect of prion biology: the ability of proteins to serve as elements of heredity.

In the baker's yeast *Saccharomyces cerevisiae*, prions create dominant cytoplasmically transmitted traits that are, in contrast to the original disease-causing prion in mammals, often advantageous to the organism (*3*). Most biochemically characterized prion proteins have a modular prion-forming domain that is highly disordered in its native state (*4*, *5*). The extreme flexibility of these domains facilitates their occasional conversion to a self-propagating conformer, which for most prions is a well-ordered fibrillar protein polymer, or amyloid. De novo prion formation appears to proceed through a high-energy oligomeric nucleus that is stabilized by interacting with, and converting, other prion proteins to the same conformation (Fig. 1A) (*4*, *6*, *7*). The elongating prion polymer is then severed into smaller, actively growing pieces by the action of protein remodeling factors such as the disaggregase Hsp104 (*8*). Lastly, the resulting fragments are disseminated to daughter cells, ensuring the stable inheritance of the self-perpetuating prion template through round after round of cell division. Indeed, prions are stable even during mating and meiosis, allowing their transmission through the germ line. Prion states are not irreversible, however. Random fluctuations in prion dissemination to daughter cells, as well as changes in the activities of remodeling proteins and other factors, can generate daughter cells with the original nonprion state (Fig. 1B).

To date, at least nine different proteins are known to form prions in *S. cerevisiae* (*3*, *9*), and an additional 18 have experimentally verified prion-forming domains (*5*). The best understood prion protein, Sup35, is a translation termination factor whose ability to form prions has been conserved for hundreds of millions of years of fungal evolution (*10*). When Sup35 switches to a prion state, its ability to function in translation is compromised, leading to increased stop codon read-through and ribosome frame-shifting (Fig. 2) (*11*, *12*). The resulting changes in gene expression have diverse phenotypic effects, including alterations in cell-adhesion, nutrient use, and resistance to various toxins and antibiotics (*12*, *13*). Importantly, these phenotypes differ in different strain backgrounds, presumably because of genetic

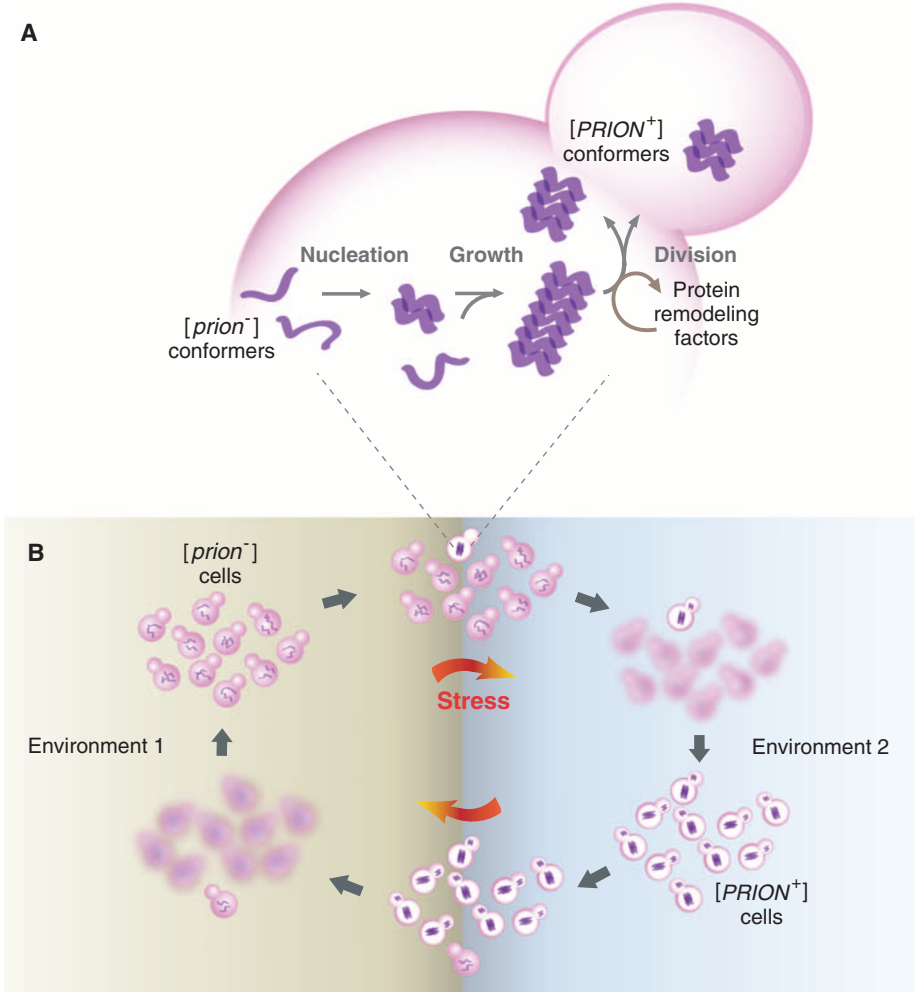

Fig. 1. Prion epigenetics. (**A**) The "life cycle" of a yeast amyloid prion. Soluble nonprion conformers in [*prion*$^-$] cells occasionally fold into an oligomeric amyloid nucleus, which then grows by sequestering additional nonprion conformers and templating their conformational conversion. The resulting prion particle divides into smaller transmissible pieces through the action of protein-remodeling factors such as Hsp104. The prion particles are disseminated to daughter cells during cell division. (**B**) Prion formation and loss are promoted by stress, and this provides a mechanism for the acquisition of heritable phenotypes in response to environmental changes. [*prion*$^-$] cells are well adapted to environment 1, but are poorly adapted to environment 2. When the environment changes, stress-induced changes in protein homeostasis result in an increased frequency of prion appearance ([*PRION*$^+$] cells) and consequently the exploration of new phenotypes. Some phenotypes revealed by prions provide a fitness advantage in environment 2, so that [*PRION*$^+$] cells survive and proliferate. The occasional loss of prion states—a process that is also increased by stress—ensures that [*prion*$^-$] cells will be available when conditions return to normal (environment 1).

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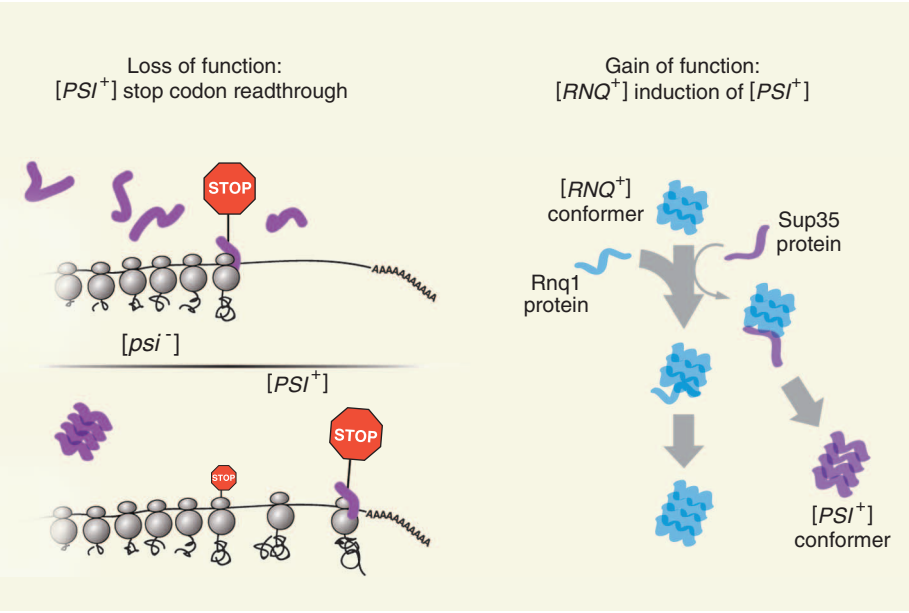

Fig. 2. Prion phenotypes can result from either a loss of function or a gain of function when the prion protein acquires its prion conformation. (**Left**) The [*PSI*$^+$] prion conformation of the translation termination factor Sup35 prevents it from associating with ribosomes. This results in the translational read-through of stop codons and corresponding C-terminal extensions that alter the activities of newly synthesized proteins. (**Right**) The Rnq1 protein, in its prion state, acquires the ability to induce other proteins, such as Sup35, to convert to their own prion states.

variation in sequences downstream of stop codons that are silent in the absence of the prion.

Prions Diversify Protein Function

Many prion phenotypes result from qualitative changes in protein function. Because function is dictated by structure, the refolding of a polypeptide into its prion form can dramatically alter the nonprion function and can even create gains of function. Aside from the ability to template their own conformational changes through homotypic interactions, some prion conformers form new interactions with other proteins. For example, the prion form of the HET-s protein in the filamentous fungus *Podospora anserina* interacts with an allelic variant of the same protein that is itself incapable of forming prions. This interaction is the basis of a self-/nonself-discrimination system that reduces the spread of parasitic cytoplasmic elements (*14*). Likewise, the prion form of the *S. cerevisiae* Rnq1 protein has the ability to interact with other prion-forming proteins. In this case, the interaction stimulates those proteins to convert to their own prion states (Fig. 2) (*8*). Another example of functionality gained in the prion state is that of the *S. cerevisiae* transcriptional regulator Sfp1. In this case, prion formation causes resistance to translation inhibitors and, remarkably, increases the cells' growth rate on rich media—phenotypes distinct from those of the nonprion state and opposite those of the genetic knockout of Sfp1 (*9*).

Prions Respond to Environmental Extremes

The way that proteins fold and interact with other proteins is very sensitive to environmental stress and the status of the protein-folding machinery. Abrupt changes in temperature, pH, and intracellular metabolites can have immediate consequences for protein folding and the regulation of protein chaperones and protein-remodeling factors. Not surprisingly then, environmental stresses also dramatically increase rates at which prions appear and disappear (*13*). The more extreme the stress, the greater the frequency of prion switching—hence, a second meaning invoked by this Perspective's title: "epigenetics in the extreme." In this way, prions connect environmental stresses with an unusual type of phenotypic plasticity that could improve an organism's ability to adapt to altered environments. When organisms experience protein homeostatic stress—which will commonly occur when they are poorly adapted to their environment—increases in protein "misfolding" and concomitant prion formation will facilitate the exploration of alternative phenotypes (Fig. 1). Indeed, we postulate that the accelerated appearance of prions in response to stress constitutes an evolved bet-hedging strategy: It allows a fraction of cells to try new phenotypes that, with reasonable frequency, prove beneficial (*3*, *15*). The self-sustaining nature of prions ensures that successful strategies are immediately heritable to subsequent generations. Prions, then, are a quasi-Lamarckian (*1*, *16*) mechanism that connects environmental conditions to the acquisition and transgenerational inheritance of new traits.

Prions Allow for the Sudden Appearance of Complex Traits

Complex evolutionary adaptations are the product of multiple interacting genetic loci (*17*). A plausible mechanism for the appearance of complex adaptations is phenotypic capacitance. Phenotypic capacitance is a property of certain biological systems that allows for the accumulation of genetic variation in a silent form, followed by its sudden stepwise release to create new phenotypes (*18*). Because prions allow cells to switch between two distinct and heritable physiological states, they provide one of the clearest examples for the reversible expression of natural genetic variation. In contrast to other mechanisms for genetically encoded stochastic phenotypic variation, such as Hsp90-buffered protein folding and variably methylated CpG islands (*19*), newly revealed prion-based phenotypes are immediately and robustly heritable. These traits can ultimately become hardwired by subsequent genetic changes, as demonstrated for phenotypes revealed by Sup35 prion formation (*12*). This observation provides experimental validation for the conjecture of West-Eberhard that in some cases genes may be followers rather than leaders in evolution (*20*, *21*).

Yeast prions are well-positioned to alter the phenotypic effects of genetic variation. The approximately two dozen prionogenic proteins discovered to date in yeast are enriched for proteins with information-processing functions, including transcription factors and RNA-binding proteins (*3*, *5*). Some, such as Swi1, Cyc8, and Sfp1, are globally acting transcriptional regulators of a large fraction of the yeast genome (*9*, *22*, *23*). Others, such as Puf2, Ptr69, and Pub1, act posttranscriptionally on the stabilities of hundreds of functionally diverse mRNAs (*24*). Because of the large number of regulatory targets of these proteins, reductions or alterations in their activities resulting from their conversion to a prion conformation can have large, and complex, phenotypic effects. Importantly, these effects also change the strength of the selective pressures that act on prion targets, resulting in these target sequences diverging at different rates when expressed under the prion versus nonprion states. As a consequence, prion-revealed phenotypes will tend to differ between genetic backgrounds (*12*). Thus, prions create phenotypic diversity on two levels: Within isogenic populations, they create distinct physiological states (prion versus nonprion), and within genetically diverse populations, they enhance the effects of genetic variation between lineages.

A Wider Range of Prion Phenomena?

In multicellular organisms, developmental signals trigger the epigenetic switches that drive cell differentiation. These switches parallel prions in

that both respond (directly or indirectly) to changes in the extracellular environment. In *S. cerevisiae*, chromatin-remodeling factors such as Swi1 and Cyc8 participate in epigenetic decisions that govern, for example, whether the cells grow as unicellular or as cohesive multicellular forms (*25*). That Swi1 and Cyc8 also form prions suggests a possible functional link between chromatin-based and prion-based regulatory strategies. In higher eukaryotes as well, prion-like switches may be involved in cell-remodeling processes. During memory formation, individual synapses must acquire a durable molecular "mark" that establishes—among the many hundreds of such marks most neurons carry—the individual long-term maintenance of that synapse. One protein contributing to this mark, neuronal cytoplasmic polyadenylation element–binding protein (CPEB), appears to undergo a prion-like conformational switch that can activate translation of synaptic mRNAs while simultaneously creating a nondiffusable self-sustaining aggregate that can act as a molecular memory (*26*). We fully expect that many such prion-like physiological switches await discovery as our abilities to characterize protein complexes and protein aggregates in vivo continue to improve.

A large array of regulatory strategies influences protein folding and may in the future prove to blur distinctions between prions and other epigenetic mechanisms for perpetuating phenotypes. Covalent modifications, including disulfide formation, phosphorylation, ubiquitination, and glycosylation, as well as protein-protein interactions (such as chaperone binding and prion templating), can all profoundly change protein-folding landscapes and/or the activity of folded proteins. All of these forms of regulation can theoretically give rise to self-sustaining heritable—that is, epigenetic—states. In fact, examples of these types of heritable factors now include an autoactivatable kinase, an autoactivatable protease, and a prion that appears to result from the interaction of two separate proteins involved in glucose signaling (*21*, *27*).

The Origins of Prions

The propensity of proteins to misfold and aggregate is probably as ancient as protein-based life forms themselves. Indeed, most polypeptides have an inherent tendency to form self-templated amyloid structures (*28*). Prion-forming proteins are unusual in having a conformational flexibility that allows access to the amyloid fold under physiological conditions (*5*, *29*). This property derives in part from a greatly reduced amino acid complexity as compared with that of globular proteins (*5*, *30*). We suggest that primordial proteins would have had similarly simple sequences, resulting in an elevated tendency to form self-perpetuating structures. Further, early biological systems would have lacked elaborate protein-folding machinery whose primary modern role is the prevention of protein aggregation. Without strong control over the important final step in the processing of gene-encoded information—protein folding—ancient polypeptides would have unencumbered access to self-perpetuating prion states. We speculate that prion formation by ancient proteins may have played a central role in the molecular evolution of early biological systems.

Our increasing awareness of prion phenomena highlights the fact that protein folding is not always uniquely specified by an amino acid sequence but instead provides a rich substrate for epigenetic determination of the map between genotype and phenotype. Beyond our speculative thoughts about early life, we suggest that prions are not simply elements of disease transmission but make distinct contributions to the flow of genetic information that are likely to profoundly influence the adaptive success, and therefore the evolution, of prion-containing organisms.

Note added in proof: The study by Derdowski *et al*. (*31*) adds another temporal dimension to the phenotypic heterogeneity conferred by prions. That the numbers of prion particles and the strength of their associated phenotypes increase as cells age suggests an accelerated exploration of alternative phenotypes among cells that have little left to lose.

References and Notes

1. O. J. Rando, K. J. Verstrepen, *Cell* **128**, 655 (2007).
2. A. Aguzzi, A. M. Calella, *Physiol. Rev.* **89**, 1105 (2009).
3. R. Halfmann, S. Alberti, S. Lindquist, *Trends Cell Biol.* **20**, 125 (2010).
4. T. R. Serio *et al*., *Science* **289**, 1317 (2000).
5. S. Alberti, R. Halfmann, O. King, A. Kapila, S. Lindquist, *Cell* **137**, 146 (2009).
6. M. Eigen, *Biophys. Chem.* **63**, A1 (1996).
7. M. M. Patino, J. J. Liu, J. R. Glover, S. Lindquist, *Science* **273**, 622 (1996).
8. I. S. Shkundina, M. D. Ter-Avanesyan, *Biochemistry (Mosc.)* **72**, 1519 (2007).
9. T. Rogoza *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **107**, 10573 (2010).
10. Y. O. Chernoff *et al*., *Mol. Microbiol.* **35**, 865 (2000).
11. O. Namy *et al*., *Nat. Cell Biol.* **10**, 1069 (2008).
12. H. L. True, I. Berlin, S. L. Lindquist, *Nature* **431**, 184 (2004).
13. J. Tyedmers, M. L. Madariaga, S. Lindquist, *PLoS Biol.* **6**, e294 (2008).
14. S. J. Saupe, *Prion* **1**, 110 (2007).
15. A. K. Lancaster, J. P. Bardill, H. L. True, J. Masel, *Genetics* **184**, 393 (2010).
16. E. V. Koonin, Y. I. Wolf, *Biol. Direct* **4**, 42 (2009).
17. D. B. Weissman, M. M. Desai, D. S. Fisher, M. W. Feldman, *Theor. Popul. Biol.* **75**, 286 (2009).
18. J. Masel, M. L. Siegal, *Trends Genet.* **25**, 395 (2009).
19. A. P. Feinberg, R. A. Irizarry, *Proc. Natl. Acad. Sci. U.S.A.* **107** (suppl. 1), 1757 (2010).
20. M. J. West-Eberhard, *Developmental Plasticity and Evolution*. (Oxford Univ. Press, Oxford, New York, 2003).
21. E. Jablonka, G. Raz, *Q. Rev. Biol.* **84**, 131 (2009).
22. Z. Du, K. W. Park, H. Yu, Q. Fan, L. Li, *Nat. Genet.* **40**, 460 (2008).
23. B. K. Patel, J. Gavin-Smyth, S. W. Liebman, *Nat. Cell Biol.* **11**, 344 (2009).
24. D. J. Hogan, D. P. Riordan, A. P. Gerber, D. Herschlag, P. O. Brown, *PLoS Biol.* **6**, e255 (2008).
25. A. B. Fleming, S. Pennings, *EMBO J.* **20**, 5219 (2001).
26. K. Si, Y. B. Choi, E. White-Grindley, A. Majumdar, E. R. Kandel, *Cell* **140**, 421 (2010).
27. J. C. Brown, S. Lindquist, *Genes Dev.* **23**, 2320 (2009).
28. F. Chiti, C. M. Dobson, *Annu. Rev. Biochem.* **75**, 333 (2006).
29. V. N. Uversky, *Front. Biosci.* **14**, 5188 (2009).
30. P. Romero *et al*., *Proteins* **42**, 38 (2001).
31. A. Derdowski, S. S. Sindi, C. L. Klaips, S. DiSalvo, T. R. Serio, *Science* **330**, 680 (2010).

32. We thank members of the Lindquist laboratory for their insightful comments on the manuscript. S.L. is an Investigator of the Howard Hughes Medical Institute (HHMI).

10.1126/science.1191081