Research News

Yeast prions and evolvability

Csaba Pál

Although much of the mystery of heredity and genetic variation has been dispelled by mendelian genetics and the modern synthesis, it is still unclear whether there are any genetic traits for facilitating the process of evolution. New work reveals that a yeast prion provides a selective advantage under adverse conditions, possibly by producing phenotypic variants. Does it also imply that the prion determinant is retained because it aids evolution?

The ubiquity of mechanisms that provide genetic variation has stimulated the argument that evolvability, the ability to generate adaptive phenotypic variation, has itself evolved in response to natural selection. This capacity might have at least three components: (1) to reduce the potential harmful effects of mutations[1,2], (2) to uncover hidden genetic variation under adverse conditions[3], or (3) to increase the rate whereby new variants arise in the population[4]. Examples include developmental modularity, heat-shock proteins and certain mutator genes, respectively.

Although these traits might have had a profound effect on adaptive evolution, it is difficult to prove that they have evolved for that purpose. This is because selection is generally regarded to be myopic – traits that do not provide an immediate advantage cannot spread in the population merely because they would be favorable in the distant future. Hence, we cannot rule out the possibility that the retention of these traits is a by-product of conservation of other, possibly unidentified, functions.

A new mechanism for evolvability has been proposed recently by True and Lindquist[5]. The authors investigated the yeast prion [PSI$^+$]. They showed that this prion has a strong and diverse effect on colony growth and morphology. Even more remarkably, the prion sometimes confers selective advantage to the colony possibly by generating new protein products.

Protein-based inheritance in yeast

Prions are generally known as infectious agents widely implicated in a variety of mammalian neurodegenerative diseases[6]. According to the most prominent model, the infectious nature of these proteins comes from the ability of the prion protein to catalyze its own propagation[7,8]. More precisely, these proteins can have at least two stable conformations: normal and prion. The prion form induces the normal protein to adopt the altered prion conformation, probably leading to the accumulation of amyloid protein aggregates. Remarkably, in addition to the prion-mediated infection, rare spontaneous events can also convert proteins to the altered prion conformation.

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This phenomenon is not restricted to mammalian diseases: certain protein-based elements of inheritance are found in baker's yeast[7–9], *Saccharomyces cerevisiae*, and in the fungi *Podospora*[9–11]. In contrast to mammalian prions, the propagation of these prions does not have fatal consequences. Rather, some results suggest that prions could have an adaptive biological function. In *Podospora*, the prion form is responsible for the control of cell fusion incompatibility.

In the case of yeast, the prion form can be stably propagated through cell division[8]. Although these experiments bring into question the tenet that DNA is the sole material for heritable changes, the evolutionary significance of these results have remained unclear[8,12]. The work of True and Lindquist gives us a clue to how a protein-based element of inheritance might affect the fate of evolutionary lineages.

Prions might confer an advantage under adverse conditions…

True and Lindquist have investigated the effects of a prion protein (Sup35) on the evolution of different strains of yeast (*S. cerevisiae*). The protein encodes a translation termination factor. The prion form [PSI$^+$] of this protein occurs in natural yeast populations, and the two different forms – prion, [PSI$^+$], and normal, [psi$^-$] – can spontaneously revert to each other. The prion reduces the fidelity of translation termination process in a heritable manner[7,8]. More precisely, the prion causes the readthrough of stop codons, leading to abnormally extended peptides (Fig. 1).

By using numerous isogenic strains differing only in the presence of the prion, the authors compared the growth characteristics of [PSI$^+$] and [psi$^-$] cells in more than 150 environments that included inhibitors affecting a variety of cellular processes. (Many of the inhibitors used in the experiments are naturally occurring antimicrobial agents.) In nearly half of the tests, marked growth differences were detected between the isogenic strains. Sometimes, the prion also had diverse effects on colony morphology. Even more remarkably, the presence of prion increased the growth dynamics in more than quarter of the tests. In a similar vein, Tuite and colleagues have previously shown that strains with the prion form exhibit enhanced stress tolerance[13]. Nevertheless, this result must be treated with caution, as this work is only partly supported by later investigations[5,14]. Furthermore, another yeast prion [URE3] consistently causes slow growth in all conditions tested so far[9].

Leaving this uncertainty aside, the work of True and Lindquist opens up the possibility that, by reducing the fidelity of protein synthesis, the prion generates enhanced variation at the protein level (as was also proposed by Inge-Vehtomov's group[15]). Some of the variants produced by the prion could permit survival under unpredictable environmental conditions. The authors speculate further that if the new phenotypic variants produced by the prion remain advantageous, then mutations eliminating the stop codons that are relevant to the favorable phenotype will be fixed in the population.

This idea is reminiscent of the concept of genetic assimilation, first proposed by Waddington in a population genetic context[16]. He argued that during the initial stage of adaptation, selection favors the genetic capacity to produce new

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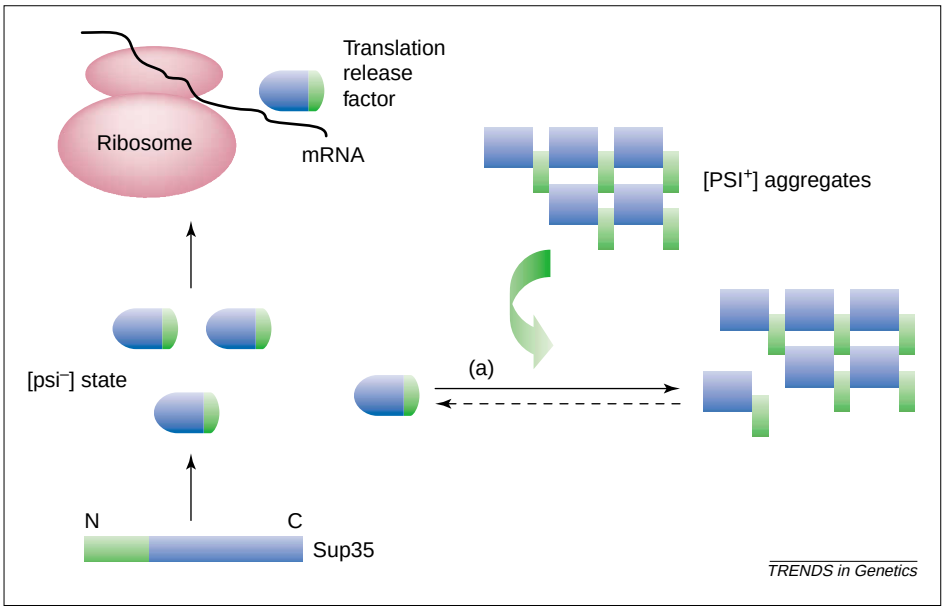

Fig. 1. A model for the prion-mediated changes of translation termination. The product of the *SUP35* gene encodes the translation release factor 3 (eRF3). The protein has two different conformations. The normal [psi$^-$] conformation is soluble, and it can spontaneously change to the [PSI$^+$] prion conformation (a). Besides these spontaneous conformation changes, [PSI$^+$] also induces the normal protein to adopt the altered prion conformation, leading to [PSI$^+$] aggregates. In [PSI$^+$] cells, less non-aggregated proteins are available, leading to inefficient termination of stop codons. The N-terminal region (or prion determinant, green) is crucial for the aggregation process, but it has no known effect on translation fidelity.

phenotypic variants, as some of them might fit the new environment. Later, mutations that constitutively express the favorable phenotypes are expected to be favored by selection, as they enable reliable transmission of the information first acquired as phenotypic variants.

...but it is unclear why they exist

As True and Lindquist freely admit, demonstrating the effects of a system on growth cannot be considered as evidence that the genetic determinants of the prion phenomenon have evolved for that purpose.

Previous work has demonstrated convincingly that the N-terminal region of the protein (prion determinant) is necessary for the maintenance of the [PSI$^+$] state of the cell: deletion of this region leads to loss of the prion followed by restored translational fidelity[17] (Fig. 1). Hence, this region seems to be essential for converting normal proteins to the prion form. Deletion of this region does not have any harmful effect on colony growth under normal conditions. This suggests that the N-terminal region of Sup35 is maintained to provide a mechanism for phenotypic variation[5].

However, there are some caveats to this interpretation of the data. We cannot rule out the possibility that the N-terminal region specifying the self-perpetuating nature of [PSI$^+$] is a side effect of other functional constraints on the Sup35 protein. In support of this theory, another group has found some evidence that the prion determinant interacts with cytoskeletal proteins[18]. Hence, the prion-determining region of the protein itself or the prion state might be involved in regulatory interactions with the intracellular structural networks.

It is also possible that [PSI$^+$] reads only specific mRNAs, so its effect might be restricted to certain genes. Indeed, numerous studies have proved that stop codon readthrough in yeast depends on the context of the stop codon and termination signals (e.g. Ref. 19). Presumably, the yeast proteins with extended C-terminal regions have distinct activities from the proteins with normal lengths. This might be crucial in a new environment. For example, the extended proteins might be less sensitive to chemical shocks. Related to this idea, the extended proteins might be involved in a general stress response pathway[13].

The availability of the complete yeast genomic sequence provides a unique opportunity to test this possibility. By examining the local sequence context surrounding stop codons in all open reading frames of the yeast genome, one could find genes that might be subject of the prion-mediated stop-codon readthrough.

Furthermore, several large reading frames are known that have a single nonsense codon embedded within them. If 3′ regions of those genes are indeed noncoding (e.g. never translated), then they are expected to evolve at an accelerated rate compared with protein coding regions (at least in prion-containing strains). By contrast, if they are functional at the protein level, at least sometimes, the difference is expected to be less profound.

How general is non-DNA-based inheritance?

Although the exact function of the prion determinant in Sup35 is unclear, some facts support the theory that protein-based inheritance might be more general than previously thought.

First, there is at least one other independently propagating prion protein in yeast, coding for a regulator of nitrogen metabolism[9]. These elements do not interfere with each other, so they can propagate independently. Furthermore, there are also some clues suggesting the existence of other yeast prion elements[20].

Second, the prion-forming potential of the *SUP35* gene is retained in related yeast species[21,22]. However, direct evidence that these species carry prions is still missing. Moreover, in contrast to the relatively frequent occurrence of the prion-containing laboratory strains of *S. cerevisiae*, prions are not observed in natural and industrial isolates of the same species[21]. These results yield a paradox: although the prion form is virtually absent in most strains, the genetic capacity to produce prions – folding properties and mechanisms – is evolutionary conserved.

Third, although mammalian and yeast prion determinants of unrelated genes show no sequence similarity, they have many properties in common. They are found in the N-terminal region of the protein, they have similar, very unusual, amino acid composition and imperfect oligopeptide repeats, suggesting that these properties might underlie prion-based inheritance[23,24]. When one considers the prion determinant of homologous genes from closely related yeast species, a similar pattern is found: although the N-terminal region evolves at an accelerated rate compared with the rest of the protein, the high glutamine and asparagine content and the charge remain largely conserved[22].

Fourth, novel prions can be created by fusion of the yeast prion determinant with mammalian genes[23]. Recent work

searching for domains with amino acid content comparable to known yeast prions has revealed numerous such domains in eukaryotes[25], but these are suspiciously lacking from prokaryotes. It would be of outstanding interest to know whether these eukaryotic proteins can also behave as elements of protein based inheritance.

'...the notion of multiple inheritance systems...should be taken seriously.'

Last, the scope of replicators that enable the propagation of phenotypic information is not restricted to prion proteins[26]. Numerous experiments show that gene expression states of certain genes can be inherited through meiosis, probably mediated through heritable chromatin-conformation changes (e.g. Refs 27,28). In agreement with previous arguments[29], theoretical works have pointed out the evolutionary significance of the phenomenon[30,31]. However, it is still unclear whether prions and meiotically heritable chromatin marks are the tip of an iceberg, or whether they represent isolated, unimportant examples. Nevertheless, the notion of multiple inheritance systems might be a big challenge for evolutionary genetics, and this possibility should be taken seriously.

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C. Pál
Collegium Budapest, Institute for Advanced Study, Szentháromság 2, Budapest, H-1014, Hungary.
e-mail: cspal@colbud.hu

DNA methylation learns to fly

Frank Lyko

For a long time, the fruit fly (*Drosophila melanogaster*) was considered the archetypal organism that lives well without DNA methylation. However, two recent reports demonstrate the presence of 5-methylcytosine in the *Drosophila* genome. This apparently contradicts numerous previous experiments that failed to detect methylated DNA. However, it is a particular combination of developmental specificity and unusual target sequences that made DNA methylation in *Drosophila* so elusive.

Cytosine methylation is essential for the proper development of several organisms, including mammals[1]. The genomes of most animals contain methylated DNA and the majority of 5-methylcytosine is concentrated at CpG dinucleotides. Usually a few percent of genomic cytosines are methylated, but the precise level can vary significantly during development. In the past, numerous experiments[2–5] established *Drosophila* as the standard organism without DNA methylation.

In addition to the fly, the genomes of *Caenorhabditis elegans* and yeast were also considered to lack DNA methylation. This led to the hypothesis that the function of DNA methylation is to reduce spurious transcription in complex genomes[6]. This 'transcriptional noise' would be negligible in organisms with a compact genome (such as *Drosophila*), and DNA methylation would therefore become dispensable[6].

DNA-methylation patterns are established, maintained and interpreted

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