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REVIEW ARTICLE

Epigenetic inheritance, prions and evolution

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Abstract. The field of epigenetics has grown explosively in the past two decades or so. As currently defined, epigenetics deals with heritable, metastable and usually reversible changes that do not involve alterations in DNA sequence, but alter the way that information encoded in DNA is utilized. The bulk of current research in epigenetics concerns itself with mitotically inherited epigenetic processes underlying development or responses to environmental cues (as well as the role of mis-regulation or dys-regulation of such processes in disease and ageing), i.e., epigenetic changes occurring within individuals. However, a steadily growing body of evidence indicates that epigenetic changes may also sometimes be transmitted from parents to progeny, meiotically in sexually reproducing organisms or mitotically in asexually reproducing ones. Such transgenerational epigenetic inheritance (TEI) raises obvious questions about a possible evolutionary role for epigenetic 'Lamarckian' mechanisms in evolution, particularly when epigenetic modifications are induced by environmental cues. In this review I attempt a brief overview of the periodically reviewed and debated 'classical' TEI phenomena and their possible implications for evolution. The review then focusses on a less-discussed, unique kind of protein-only epigenetic inheritance mediated by prions. Much remains to be learnt about the mechanisms, persistence and effects of TEI. The jury is still out on their evolutionary significance and how these phenomena should be incorporated into evolutionary theory, but the growing weight of evidence indicates that likely evolutionary roles for these processes need to be seriously explored.

Keywords. epigenetics; epigenetic inheritance; evolution; prions.

Introduction

The word epigenetics—'the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being'—was coined by Conrad Waddington (Waddington 1942), largely as a conceptual tool to understand development better in terms of both the actions of genes, and cellular and organismal processes and mechanisms crucial to development. This was at a time when the disciplines of genetics and embryology proceeded largely in parallel and aloof from each other, and Waddington felt that, to understand the complexities of embryonic development as well as the evolution of the developmental mechanisms that gave rise to different forms, a more integrated approach that bridged the gap between genetics and embryology was required. The term epigenetics over the following several decades appeared rather sparsely in publications or research programmes. However, in the past two decades or so, an extremely active epigenetics research field has grown tremendously, albeit with a somewhat different meaning being generally attributed to the term epigenetics. By and large, the term is currently used to imply that altered phenotypic states—be they at the level of patterns of gene expression, cell differentiation or some other aspect of organismal phenotype—can be transmitted through cell divisions at a nongenetic level, i.e. without any changes in DNA sequence; such epigenetic inheritance is less stable than DNA-based inheritance, and generally reversible. The bulk of research in the field—93% by one estimate (Burggren 2016)—deals with how epigenetic mechanisms, primarily at the level of chromatin modifications, DNA methylation and the action of small noncoding RNAs, regulate states of gene expression that are transmitted mitotically. These studies are in the contexts of development, cell differentiation, disease states arising from errors in epigenetic modifications etc. However, an ever increasing number of instances of epigenetic inheritance is also being described in which traits are transmitted across generations, i.e. through meiosis. Such transmission is generally referred to as transgenerational (as opposed to intragenerational) epigenetic inheritance. The focus of

this review is on transgenerational epigenetic inheritance (hereafter referred to as TEI) and its possible significance for evolution, with a special focus on a possible role for prions. Although epigenetic phenomena are not restricted to eukaryotes, this discussion is limited to this group. Further, the dynamics and evolutionary implications of TEI in sexually and asexually reproducing species are likely to be different, but the discussion here is confined to sexually reproducing organisms (though yeasts like *Saccharomyces cerevisiae* can alternate between sexual and asexual modes of propagation).

In a somewhat trivial sense (from the point of evolutionary mechanisms), intragenerational epigenetic inheritance has been crucial during evolution: without the prior evolution of these epigenetic mechanisms that ensure stable maintenance of gene expression patterns during differentiation and development to confer cell memory, it is unlikely that complex multicellularity could have evolved. Apart from that, intragenerational epigenetic inheritance processes might also influence evolution in additional ways, e.g. by regulating the activity/inactivity of transposable elements. Transposable elements are thought to have contributed to evolution through effects on expression of nearby genes—thus sometimes giving rise to novel expression patterns (e.g. see Coen *et al.* 1986; Wessler 1988; White *et al.* 1994; Bureau *et al.* 1996; Kumar and Bennetzen 1999; Lippman *et al.* 2004)—or by causing genomic rearrangements that could have a variety of evolutionary consequences, including in promoting speciation (reviewed in Fedoroff 2012). Differences in epigenetic marks could also lead to 'epigenetic incompatibility', creating a reproductive barrier that could promote speciation (Tarutani *et al.* 2010; Durand *et al.* 2012; Lafon-Placette and Köhler 2015).

Neo-Darwinian evolutionary theory has hitherto taken little account of epigenetic phenomena, though there have been challenges over the decades to the neo-Darwinian viewpoint (e.g. Gould 1977; Ho and Saunders 1979). To quote Ho and Saunders: 'Contrary to the neo-Darwinian view, we point out that the variations of the phenotype, on which natural selection could act, do not arise at random; they are produced by interactions between the organism and the environment during development. We propose, therefore, that the intrinsic dynamical structure of the epigenetic system itself, in its interaction with the environment, is the source of nonrandom variations which *direct* evolutionary change, and that a proper study of evolution consists in the working out of the dynamics of the epigenetic system and its response to environmental stimuli as well as the mechanisms whereby novel developmental responses are canalized.'

With the rapidly expanding understanding of epigenetic mechanisms and the accumulating evidence for TEI, there has been some strong advocacy in recent years of an expanded view of evolutionary theory, sometimes called the extended evolutionary synthesis or EES (e.g. Pigliucci 2007; Danchin *et al.* 2011; Jablonka 2012, 2013; Jablonka and Lamm 2012; Skinner 2015). The debate between advocates of an overhaul of evolutionary theory and defenders of the 'status quo' recently featured in the pages of *Nature* (Laland *et al.* 2014), and a discussion of the possible evolutionary importance of epigenetic mechanisms in *Nature Reviews in Genetics* (Grossniklaus *et al.* 2013). The new subarea of environmental epigenetics, which is seeing a stream of reports on environmentally elicited TEI in plants as well as animals has further stimulated the debate. Numerous good reviews and discussions have appeared in recent years (Daxinger and Whitelaw 2012; Jablonka 2012, 2013; Grossniklaus *et al.* 2013; Koonin 2013; Lim and Brunet 2013; Duncan *et al.* 2014; Heard and Martienssen 2014; Schmitz 2014; Burggren 2016; Verhoeven *et al.* 2016). I shall provide a cursory overview of TEI in plants and animals before turning to a TEI phenomenon so far verified only in fungi, namely that of protein-based epigenetic inheritance through prions. While the possible role of prions in evolutionary adaptation has been energetically debated in the prion research community, it has not elicited much discussion in wider debates on epigenetic inheritance and evolution.

That TEI does occur, though in most instances the mechanisms remain poorly understood, is borne out by an ever growing list of instances. The fact that epigenetically determined phenotypic changes can not only be induced by the environment—generally in response to environmental stress—but that the altered phenotypes can get passed on to subsequent generations, has further whetted interest in the possible adaptive value of TEI. Much of the debate is about whether and under what conditions TEI could be adaptive, especially under altered or fluctuating environmental conditions.

Epigenetic inheritance

The best understood mechanisms of epigenetic inheritance are based on modifications to the way DNA is packaged in chromatin to affect gene expression. An array of covalent modifications occurs on histones, which form nucleosomes at the first level of chromatin structure. Through acetylation, methylation, phosphorylation, ubiquitination and a number of other modifications carried out on specific histone residues, regions of chromatin are marked in ways that determine structure and accessibility to the nuclear machinery. Depending on the kinds and combinations of histone modifications, chromatin may either be in an 'open' structure in which the DNA is accessible to regulatory proteins such as transcription factors, or in a 'closed' state in which the chromatin is further packaged in more condensed higher order structures which prevent interaction with most regulatory proteins. The covalent modifications can also be removed, so that chromatin structure is dynamic and can be modified in response to developmental

or environmental signals. In addition to covalent histone modifications, in some groups of multicellular eukaryotes (including plants and mammals), DNA can also be methylated on cytosine residues at CpG or CpXpG sites. DNA methylation and histone modifications act in concert to determine the structure and functional state of chromatin. Patterns of both are transmitted to daughter cells after DNA replication. The finding that DNA methylation patterns can sometimes also be transmitted to progeny was unexpected, since most DNA methylation marks are removed during two waves of demethylation, first during gametogenesis and subsequently in very early embryonic development, to be re-established *de novo* later during embryogenesis. However, it appears that some methylation marks escape erasure, and little is known about how this happens. In addition to histone modifications and DNA methylation, a number of noncoding RNAs, many of which are also involved in chromatin remodelling, are also implicated in epigenetic inheritance.

Transgenerational epigenetic inheritance

TEI frequently appears to involve, at least in the cases where something is known about the mechanism, transmission of modified DNA methylation patterns or of RNA molecules to the progeny, by means of which a phenotype is transmitted. An early example of this was in the toad flax plant *Linaria vulgaris*. The plant normally has bilaterally symmetrical flowers, but an epigenetic variant (an 'epiallele') produces radially symmetrical flowers (Cubas *et al.* 1999). This was shown to be associated with hypermethylation in the promoter region of the *CYCLOIDEA* gene (Mathieu *et al.* 2007). The epiallele is stable through many generations. Subsequently numerous other examples of stably transmitted epialleles have been described (e.g. Manning *et al.* 2006; Durand *et al.* 2012; reviewed in Weigel and Colot 2012), some of which are 'pure' (i.e. with no detectable associated genetic basis), while some seem to be related to specific DNA sequences that appear to 'trigger' their generation.

Paramutations are another odd epigenetic phenomenon in plants (Chandler 2007; Hollick 2012). A number of examples are known in which a paramutagenic allele can convert a paramutable allele to the paramutagenic state when they are together in a heterozygote. Thus in the b1 locus of maize, which is involved in regulating anthocyanin biosynthesis, a paramutagenic b′ allele that results in light pigmentation, can convert another allele, B-I, to b′. B-I when homozygous has a darker pigmentation phenotype. Once a B-I allele is converted to b′, it in turn becomes paramutagenic. The paramutagenic property of b′ was found to reside in a set of seven tandem 853 bp repeats about 100-kb upstream of b1. In b′ these are hypermethylated and cause lower transcription of b′. b′ can convert B-I by bringing about hypermethylation of the B-I repeats (Stam *et al.* 2002). Neutral b1 alleles which lack the repeats or have less than three are not paramutable. In general, paramutation in maize appears to occur by a *trans*-acting siRNA mechanism (Alleman *et al.* 2006) involving production of a noncoding RNA (usually transcribed from nearby repeat sequences or transposable elements) and requiring RNA-dependent RNA polymerase activity and a number of other proteins such as the chromatin remodeller RMR1 and the DNA binding protein CBBP. A number of other examples of paramutation are known, with differences in the nature of associated *cis*-acting sequences required for paramutagenicity, the efficiency of paramutation, and the stability of the paramutagenic epialleles, both in plants and animals (for recent discussions see Gabriel and Hollick 2015; Hövel *et al.* 2015; Pilu 2015; Ronsseray 2015; Sapetschnig *et al.* 2015).

In animals, an early report of TEI was about inheritance of the mouse A^{vy} agouti allele (Morgan *et al.* 1999) followed by another report from Whitelaw's lab of TEI at the Axin locus (Rakyan *et al.* 2003). The Axin-fused allele ($Axin^{Fu}$) which causes a kinked tail phenotype in some epigenetic states has a retrotransposon insertion at the Axin locus. Variability in the kinked tail phenotype of $Axin^{Fu}$ mice was shown to be associated with different levels of DNA methylation in the retrotransposon. The progeny mice showed similar DNA methylation levels (hyper methylation or hypomethylation) and similar phenotypes to the parents.

Numerous papers report TEI induced in mammals under stresses such as environmental toxins like the fungicide vinclozolin, the pesticide methoxychlor, dioxin, di-(2-ethylhexyl) phthalate, the plastics additive bisphenol A and a number of others (Anway *et al.* 2005; Crews *et al.* 2012 and many others), as well as nutritional deprivation or drastically skewed diets (Cropley *et al.* 2006; Ferguson-Smith and Patti 2011; Ost *et al.* 2014; Radford *et al.* 2014; Yan 2014). These are only a few of the now numerous examples of TEI. While details of precise mechanisms of transmission and persistence of the effects are likely to vary considerably, these phenomena are of great intrinsic interest (recently reviewed or discussed in Feil and Fraga 2012; Iwasaki and Paszkowski 2014; Kinoshita and Seki 2014; Szyf 2015). An early report of an environmental effect eliciting a defensive (adaptive) response that was transmitted to the next generation (Agrawal 2002), described how in wild radish plants (*Raphanus raphanistrum*), caterpillar herbivory or jasmonic acid treatment induced resistance in the progeny. Useful reviews and discussions of the evidence for TEI in plants have appeared at regular intervals (Paszkowski and Grossniklaus 2011; Pecinka and Mittelsten Scheid 2012; Schmitz and Ecker 2012; Weigel and Colot 2012; Heard and Martienssen 2014; Turck and Coupland 2014; Groot *et al.* 2016) as well as in animals (Daxinger and Whitelaw 2012; Lim and Brunet 2013;

Heard and Martienssen 2014; Whitelaw 2015). Where epigenetically determined traits that appear to benefit the organism can be transmitted to progeny (e.g. Crews *et al.* 2012; Holeski *et al.* 2012; Vandegehuchte and Janssen 2014; Herman and Sultan 2016; Lankinen *et al.* 2016; Verhoeven *et al.* 2016), it is tempting to believe that this is bound to have adaptive value. That environmental stress (or other cues) can induce epigenetic changes is well-documented (Bastow *et al.* 2004; Sung and Amasino 2004; Yan 2014 and reviews cited above). For an excellent short review of stress-induced epigenetic changes in plants, see Probst and Mittelsten Sheid (2015). There is strong evidence that some of these changes can indeed be transmitted to progeny (Molinier *et al.* 2006; Seong *et al.* 2011 and references in reviews cited above), and on the face of it some of the phenotypic effects of such changes appear to be likely to have adaptive value.

A striking feature common to many (though by no means all) different TEI phenomena is the involvement of transposable elements (TEs). The genomes of complex multicellular organisms are replete with TEs, which may comprise 50% of the genome in mammals, or as much as 80% in plants like maize. Various mechanisms seem to have evolved which prevent TEs from causing genomic catastrophe through frequent mobility, but when transpositional events do occur, they can have profound consequences. The 'activities' of transposable elements are remarkably sensitive to a variety of environmental stresses (McClintock 1984; Fedoroff 2012). Stress signalling can lead to epigenetic changes such as hypomethylation in the chromosomal neighbourhood of TEs, which in turn can have a variety of consequences. A direct effect could be through effects on the expression of nearby genes through TE sequences with enhancer activity, which may be transmitted to progeny. Hypomethylation and the resulting changes in chromatin structure also renders TEs recombinationally active, which can result in chromosomal rearrangements and the various consequences of such rearrangements. More indirect effects of epigenetic modifications may also occur through transcription from TEs. When TE-encoded transposases are expressed TEs are mobilized, with outcomes such as changes in chromosome organization and/or gene expression. Apart from the production of TE-encoded proteins, transcription from TEs may also lead to epigenetic changes in nearby genes through small RNA dependent pathways such as the RNA-dependent DNA methylation pathway in plants or the piRNA pathway in animals that are implicated in paramutation. Transposable elements may thus play a significant role in triggering or mediating epigenetic changes that can be carried over to subsequent generations, a role which is particularly interesting because of the linkage to environmental stress.

While the environmental induction of epigenetic changes with adaptive value to stress conditions—and in many cases their transgenerational inheritance—is well established, it is not entirely obvious that these epigenetically altered traits are adaptive on a longer evolutionary time scale. The persistence and stability of traits transmitted through TEI varies greatly in different cases. Under what conditions and in what ways could TEI be adaptive?

One proposed way that TEI could be adaptive is simply by providing a time window of survival under stress, during which environmental conditions might 'soften'. TEI would act as a bet hedging strategy. Under persistent stress conditions, genetic mutations could eventually arise to fix epigenetically determined favourable traits. Karpinets and Foy (2005) have surveyed evidence on epigenetic and genetic alterations occurring during tumourigenesis, and propose that tumour cells accumulate epigenetic changes at tumour suppressor genes (hypermethylation) and protooncogenes (hypomethylation), which 'prime' these loci for mutations that lead to genetic fixation of their stress survival phenotype. While tumour cells may not be a good model for organismal adaptation, a more general possibility is that cytosine methylation may lead to higher mutation rates at the methylated residues, thereby enhancing the possibility of genetic fixation in hypermethylated regions. Somewhat stronger evidence for genetic fixation of epigenetically originated phenotypes comes from work on the budding yeast [PSI^+] prion, where, in some cells, [PSI^+]—induced phenotypes were found to persist in the absence of the prion (True *et al.* 2004). It is worth pointing out that genetic fixation is not necessarily dependent on *de novo* mutations, but could also arise in the first instance through recombination.

Since environmentally induced epigenetic traits that can be transmitted are likely to be present in a significant proportion of the progeny, there would be a much larger pool of individuals among which favourable genetic variations could be selected. It has also been suggested that under conditions of rapid environmental change or fluctuation, strong selection pressure favouring rare genotypes could result in depletion of genetic variation in the population, and that environmentally induced adaptive epigenetic changes could provide a 'safe' bet-hedging strategy which would protect genetic variation, or could aid survival by exposing cryptic genetic variation or even facilitate new mutations (O'Dea *et al.* 2016).

Modelling studies of the possible evolutionary outcomes of TEI, considering factors like stability of the epigenetic trait; magnitude of the selection pressure; reliability of epigenetic variation-inducing environmental cues in 'predicting' or anticipating the future environment; instability or heterogeneity (spatial as well as temporal) of the environment; and the cost of maintaining epigenetic plasticity suggest that TEI could be adaptive under some conditions but not others (Herman *et al.* 2013; Furrow and Feldman 2014; Schlichting and Wund 2014; Uller *et al.* 2015; Chisholm *et al.* 2016; Gòmez-Schiavon and Buchler 2016; Kronholm and Collins 2016).

Prions as mediators of epigenetic inheritance

Prions were first identified in mammals, through the contributions over many years of a number of workers, as infectious protein particles that were the causative agents of transmissible spongiform encephalopathy (for an interesting historical account see Zabel and Reid 2015). Subsequently prions were identified in yeasts and fungi; the first yeast prion to be 'proposed' was [URE3] (Wickner 1994). [URE3] had been known for years for its odd behaviour, showing non-Mendelian inheritance and being thought of as some kind of extrachromosomal element (Lacroute 1971). The identification of [URE] as a yeast prion was followed by that of a number of others (Patino *et al.* 1996 and references below). [PSI^+], like [URE3], had first been reported earlier as an extrachromosomal element (Cox 1965). Of all yeast prions, it remains the best characterized and most intensively studied. In 2000, True and Lindquist (2000) published intriguing results describing a plethora of phenotypic effects under a variety of conditions, of the presence of this prion. These effects were strongly dependent on the yeast strain (read genetic background), and were variably advantageous or disadvantageous to growth/survival; under the same conditions, the presence of the PSI prion (i.e. the [PSI^+] state) conferred growth advantage in some strains while being disadvantageous in others. A follow-up paper (True *et al.* 2004) extended these findings. They were interpreted to mean that this prion might constitute an evolutionarily significant means of adaptation to fluctuating environments that could function as a 'bet-hedging' mechanism by exposing cryptic genetic variation to generate novel, potentially adaptive phenotypes. It is worth emphasising that, firstly, while the rate at which [PSI^+] arises spontaneously is comparable to mutation rates under relaxed growth conditions, it is higher under conditions of stress (Tyedmers *et al.* 2008; Chernova *et al.* 2014), when novel phenotypes are more likely to be advantageous; secondly, that the highly pleiotropic effects of [PSI^+] may 'expose' a range of phenotypes that would otherwise require multiple mutations in multiple genes; and lastly, that the prion 'switch' is reversible, with a sufficiently high frequency of prion loss to allow reversion to the prion-less state under conditions of rapid environmental fluctuation. The third yeast prion to be described was [PIN^+] (Derkatch *et al.* 2001), which turned out to be the prion form of the Rnq protein (Sondheimer and Lindquist 2000) (though some other protein aggregates can also act like [PIN^+]). While the function of the normal nonprion form of Rnq is still unknown, the [PIN^+] prion appears to be essential for the *de novo* generation of most other prions, presumably through some physical cross interactions.

The discovery of these three prions was followed by that of at least 25 more, largely as a result of a systematic search for novel prions by Lindquist and coworkers (Du *et al.* 2008; Alberti *et al.* 2009; Brown and Lindquist 2009; Patel *et al.* 2009; Halfmann and Lindquist 2010; Rogoza *et al.* 2010; Halfmann *et al.* 2011, 2012; Suzuki *et al.* 2012; Holmes *et al.* 2013, Jarosz *et al*. 2014). A useful concise, if already slightly outdated, overview is that of Crow and Li (2011), while a very comprehensive review can be found in Liebman and Chernoff (2012).

Prion structure, formation and transmission

Prion-based inheritance is unique among epigenetic phenomena in that, unlike other known epigenetic mechanisms, it occurs entirely at the protein level rather than through direct or indirect modification of gene expression. Common to all prions is an inherent tendency to adopt an alternative amyloid protein structure to form fibrous aggregates. *De novo* prion formation appears to occur stochastically with frequencies dependent upon the particular protein (which may be dependent both upon the sequence of the protein and its cellular levels). Further, the frequency at which prions spontaneously arise may be influenced by the presence of other prions as well as by environmental conditions such as various kinds of stresses like high temperature, osmotic stress, oxidative stress and the unfolded protein response (Cox *et al.* 1988; Chiti and Dobson 2006; Tyedmers *et al.* 2008; Chernova *et al.* 2011; Newnam *et al.* 2011; Holmes *et al.* 2013; Doronina *et al.* 2015). Prions (in common with some nonprion-forming proteins) form highly aggregated fibres rich in amyloid β sheets. What distinguishes prions from other kinds of amyloid protein aggregates is their ability, once formed, not only to seed the conversion of 'normally' folded protein into prions by acting as templates for their aggregation into amyloid fibres (or possibly by diverting newly synthesized polypeptide chains into an alternative folding pathway), but also the segregation of prion particles to daughter cells after cell division. Since aggregation into prions mostly results in loss of activity of the protein, the prion state often (but not always: see Derkatch *et al.* 2001; Rogoza *et al.* 2010; Suzuki *et al.* 2012; Holmes *et al.* 2013) results in phenotypes resembling those of loss-of-function mutations. The transmission of prion particles to daughter cells is by no means automatic, but requires the action of a number of stress proteins such as Hsp104 (which severs large aggregates into smaller ones that can be transported to daughter cells) (Chernoff *et al.* 1995) and several other heat shock proteins such as Hsp70 and Hsp40 (Rikhvanov *et al.* 2007; Shorter and Lindquist 2008; Romanova and Chernoff 2009; Reidy and Masison 2011). It is an efficient process, because of which prion inheritance shows characteristic non-Mendelian segregation ratios (4:0 in *Saccharomyces cerevisiae*). However, prion transmission is not perfectly efficient, allowing for rare prion loss, an important property in considerations of the possible adaptive value of prions.

The rates at which prions arise *de novo* and are lost vary widely between prions (from $\sim 10^{-2}$ to 10^{-7}), as well as under different environmental or physiological conditions. Both prion induction and loss are affected by numerous factors such as a variety of stresses (temperature, osmotic, oxidative etc.), concentration of the prion-forming protein, as well as interactions with other cellular components such as other (heterologous) intracellular protein aggregates, chaperones and heat-shock proteins, the actin cytoskeleton, and components of the ubiquitin–proteasome system (reviewed in Chernova *et al.* 2014). Thus, the frequencies of prion switching can vary over orders of magnitude under different conditions. In the case of [*PSI*$^+$], oxidative stress has been shown to cause oxidation of methionine residues, which increases the propensity of the protein to fold into the prion conformation (Sideri *et al.* 2011; Doronina *et al.* 2015). In the remarkable case of the [*MOT3*$^+$] prion, induction and loss of the prion are strongly dependent on physiological conditions. The prion arises at very high frequency in the presence of ethanol or during growth on proline as nitrogen source, and is lost under hypoxic conditions. In this case loss of the prion seems to be the rather straightforward result of inhibition of expression of the *MOT3* gene by hypoxia.

In almost all prions characterized so far, the ability to form the prion aggregate and recruit soluble protein into the aggregate depends on a prion-forming domain (with stability being affected by additional discrete sequences). These domains appear to require highly skewed Q/N rich amino acid compositions and positioning of amino acids rather than specific conserved sequences. Experimental work and bioinformatic analyses suggest that numerous proteins harbour sequences capable of forming prions besides the demonstrated examples (Ross *et al.* 2004; Alberti *et al.* 2009; Halfmann *et al.* 2011; Toombs *et al.* 2011; Angarica *et al.* 2013; Lancaster *et al.* 2014; MacLea *et al.* 2014; Bondarev *et al.* 2015). In addition to sequence requirements for prion formation, almost all yeast prions require the presence of the [*PIN*$^+$] or [*RNQ*$^+$] prion for *de novo* formation (but not for subsequent maintenance). However, the same prion forming domain can give rise to different variant forms (often referred to as prion 'strains'). Different strains of a prion differ structurally, in the size and stability of the aggregates formed, in the efficiency of transmission of the prion to daughter cells (and hence its stability and persistence), in the relative amounts of the soluble and aggregated forms of the protein, and in the weaker or stronger phenotypes they engender (reviewed in Liebman and Chernoff 2012). In this sense, prion strains are analogous to allelic series of a gene.

Prions in wild strains of yeast

In light of the intriguing phenotypic effects of the [*PSI*$^+$] prion and the discovery of additional prions (and perhaps of criticisms of the idea that prions could be functional elements rather than merely misfolded 'diseased' proteins occurring in yeast populations only in laboratory culture conditions), Lindquist and coworkers undertook a systematic search for prions in some 700 wild strains of yeast (Alberti *et al.* 2009; Halfmann and Lindquist 2010; Halfmann *et al.* 2010, 2012). This work uncovered a number of novel prions that were present at appreciable frequencies (in about 1/3 of all wild strains of yeast isolated from a variety of ecological contexts), and, in a number of these strains, curing them of prions resulted in altered phenotypes. Phenotypes associated with some of these prions were strongly suggestive of beneficial effects of the prion state, such as resistance to acidic conditions in a strain isolated from white wine, or resistance to the antifungal agent fluconazole or the DNA damaging agent 4-nitroquinoline-1-oxide (4-NQO). The emerging picture is that free-living populations of yeast harbour a variety of prions, strongly biased towards regulators of cell metabolism such as transcription factors and signalling proteins (Halfmann and Lindquist 2010). The [*PSI*$^+$] prion and some of the other interesting prions that have turned up in the past few years are briefly described below.

[PSI]

[*PSI*$^+$] is the prion state of the protein Sup35, which together with another protein, Sup45, normally functions in translation termination at stop codons. In the prion state, the amount of functional soluble Sup35 available for translation termination is greatly reduced because of formation of the insoluble prion aggregate, resulting in compromised translation termination. [*PSI*$^+$] thus acts much like a nonsense suppressor, causing read-through of stop codons. This gives rise to a remarkable range of altered phenotypes (True and Lindquist 2000; True *et al.* 2004). The [*PSI*$^+$]-dependent phenotypes are strongly dependent on genetic background, presumably reflecting underlying cryptic genetic variation that is not manifested phenotypically in cells lacking the prion (designated [*psi*$^-$]). Most of the phenotypes were shown to be due to nonsense suppression, and as many as 1/3rd of them were beneficial for growth or survival under specific conditions. However, [*PSI*$^+$] may have additional effects on phenotype independent of nonsense suppression (Baudin-Baillieu *et al.* 2014).

Lindquist and coworkers have proposed that the properties of [*PSI*$^+$] and a number of other prions (spontaneous *de novo* generation and loss) could confer adaptive advantage to yeast populations living in unstable fluctuating environments (True and Lindquist 2000; True *et al.* 2004; Shorter and Lindquist 2008; Halfmann *et al.* 2010). Under conditions unfavourable for [*psi*$^-$] cells, the few [*PSI*$^+$] cells that would arise spontaneously would ensure survival of the (presumably largely clonal) population by

providing new phenotypes due to the 'exposure' of cryptic genetic variation at many loci. If the environmental change was long-lasting, such [PSI^+]-dependent phenotypes could get fixed genetically in the course of time. In unstable, rapidly fluctuating environments, flipping between [PSI^+] and [psi^-] states could provide a non-genetic bet-hedging strategy that would not necessarily require genetic fixation. Mathematical modelling suggests that switching between [psi^-] and [PSI^+] states at the observed rates could be evolutionarily beneficial even for small selective advantages (King and Masel 2007; Griswold and Masel 2009; Lancaster *et al.* 2010). Tantalisingly, the switching frequency—the rates at which [PSI^+] arises and is lost—is not invariant but is enhanced under stress conditions (Cox *et al.* 1988; Tyedmers *et al.* 2008; Chernova *et al.* 2011; Newnam *et al.* 2011; Holmes *et al.* 2013), where some novel phenotypes might carry greater benefit.

[MOT3$^+$]

[$MOT3^+$] is the prion form of the transcriptional regulator Mot3, which is part of a complex system of regulation of the important 'life-style' choice between single-celled yeast-style growth and multicellularity (Holmes *et al.* 2013). The non-prion form of the protein functions as a co-repressor of several genes, including the Flo11 gene which is crucial for invasive (filamentous) growth and other kinds of multicellular interactions. The [$MOT3^+$] prion had effects on multicellular interactions that depended on the genetic background of the strain, causing filamentation and invasive growth in some strains, altered colony morphology in others.

In addition, Mot3 affects other metabolic processes and is involved in regulating the expression of genes required for anaerobic growth (repressing them during aerobic growth). Fascinatingly, the induction and loss of the [$MOT3^+$] prion state is highly sensitive to metabolic conditions: the prion is induced by ethanol and lost under hypoxic conditions which follow utilization of ethanol. Thus when cells utilize ethanol for non-fermentative growth, the protein in the [$MOT3^+$] prion form does not repress genes required to be expressed during this phase of growth. As ethanol is depleted and hypoxic conditions come to prevail at the end of this phase of growth, the prion is lost and subsequently the non-prion protein becomes available to function as a repressor of genes expressed during aerobic growth. It is difficult to resist the conclusion that switching between the prion and non-prion states constitutes a mechanism for regulating metabolism.

[MOD$^+$]

The Mod5 protein is a yeast tRNA isopentenyl transferase that modifies a base in the anticodon loop of tRNAs. Suzuki *et al.* (2012) reported that, despite lacking a Q/N-rich region, Mod5 is able to adopt a prion conformation [MOD^+]. In prion cells there is decreased tRNA isopentenyl transferase activity but increased ergosterol biosynthesis, conferring resistance to antifungal agents like fluconazole, ketoconazole, and clotrimazole that inhibit ergosterol synthesis. [MOD^+] cells grown in the presence of fluconazole retained the prion, while cells progressively lost the prion in the absence of the drug. In addition, [MOD^+] cells also showed greater tolerance for the microtubule inhibitor nocodazole.

GAR

[GAR$^+$] (for 'resistant to glucose-associated repression'), first described by Brown and Lindquist (2009), is a thoroughly unorthodox prion in that the prion state depends upon two proteins, Std1 and Pma1, encoded by different genes. Further, unlike all other prions characterized to date, its transmission does not require Hsp104 and is unaffected by the presence of other prions, and [GAR$^+$] does not appear to form an amyloid structure. Curiously, although overexpression of the *STD1* gene increases the frequency of [GAR$^+$] formation, deletion of either the *STD1* or the *PMA1* gene alone does not cure the prion, though deletion of *PMA1* together with the presumed prion-forming domain of *STD1* does. [GAR$^+$] is involved in regulation of carbon source utilization: in the prionless state, repression of utilization of alternate carbon sources is operative, but [GAR$^+$] cells become resistant to the otherwise stringent glucose repression, allowing them to utilize alternate carbon sources even in the presence of glucose (Brown and Lindquist 2009; Jarosz *et al.* 2014a). Amazingly, [GAR$^+$] formation can be triggered by signalling by a bacterium, *Staphylococcus hominis*, in the environment (Jarosz *et al.* 2014b)—an arrangement that appears to be favourable to both the yeast and the bacterium.

[SWI$^+$]

[SWI^+] is the prion form of the Swi1 protein (Du *et al.* 2008; Alberti *et al.* 2009), a subunit of the Swi/Snf chromatin remodelling complex required for induction of a number of genes such as those required for mating type switching and sucrose utilization (whence the name Swi/Snf). The [SWI^+] prion causes loss of flocculation (*FLO*) gene expression, resulting in loss of the ability to form multicellular filaments. Further, a number of other proteins such as Mss11, Msn1 and Sap30 that act as regulators of *FLO* gene expression, also undergo conformational changes rendering them inactive in [SWI^+] cells. Mss1 in these cells can form prion-like aggregates that persist even after loss of [SWI^+].

[OCT$^+$]

[OCT^+] is the prion form of another global transcriptional regulator, Cyc8, which together with Tup1 forms a repressor complex. [OCT^+] cells show increased flocculence and altered carbon source utilization, such as the ability to grow on lactate because of constitutive invertase activity.

Fungal prions: disease or beneficial agents?

In this brief account of the properties of a few among the growing number of prions that have been identified, I have attempted to give a flavour of the variety of phenotypic effects prions can exert, and the possibility that some of these have been evolutionarily co-opted to serve cellular responses to environmental conditions. Besides the spirited advocacy by Lindquist and coworkers of a functional role for at least some prions in metabolism and evolution (True and Lindquist 2000, 2004; Shorter and Lindquist 2008; Holmes *et al.* 2013; Newby and Lindquist 2013), some others have also argued the case for their functional significance (e.g. see Tuite and Serio 2010). Tuite (2015) has pointed out that in a formal (though mechanistically very different) sense, fungal prions act much like paramutations in plants. While many of their effects, such as lower fidelity of translation termination in [PSI^+], and the pleiotropic phenotypes engendered by them are likely to be maladaptive in many situations, what is of interest is that some prion-associated phenotypes could confer survival advantage under specific conditions. Particularly interesting is the fact that the frequency at which some prions arise and are lost is sensitive to the environment, with enhanced rates of acquisition and loss under stress. In the unstable, variable environments that microorganisms typically encounter, prions could therefore provide a bet-hedging mechanism for survival under altered conditions. Apart from the various phenotypes they give rise to, the existence of a plethora of prions in wild strains of yeast; the conservation of prion-forming domains of proteins among different species over a time scale of the order of a 100 million years; the effects of environmental conditions on prion switching rates; and the existence of what appears to be a fairly elaborate cellular machinery that brings about the partitioning of prions to daughter cells, all suggest that the capacity to generate prions may have been conserved (and elaborated) during evolution. There is no general agreement that prions have functional significance (e.g. see Wickner *et al.* 2011; Edskes *et al.* 2014; Wickner 2016, who have unflaggingly argued that, with the exception of the *Podospora anserina* [*Het-s*] prion, fungal prions represent no more than a disease state due to protein misfolding that occurs sporadically at frequencies too low to be of adaptive significance), but the possibility that prions may have functional roles and could promote adaptation under some conditions merits very serious consideration. Perhaps evidence from lab evolution experiments will eventually contribute to a better understanding.

Lab evolution experiments are, of course, tricky to design, but can provide a wealth of information about changes occurring in populations in the course of adaptation under imposed selection pressures. By far, the biggest such undertaking has been the *E. coli* long term evolution project, which has been running since 1988, spanning over 50,000 generations (for an interesting account, see Fox and Lenski 2015). Budding yeast shares many of the features that make *E. coli* ideal for such evolution experiments—short generation time, huge numbers, sophisticated genetic and molecular techniques, the ability to freeze away and store population samples at various time points of the experiment, and so forth. Given the number of different prions with the range of genetic background-dependent phenotypic effects, a dauntingly large range of experimental evolution experiments await doing. To determine effects of prions on adaptation, a variety of selection regimes would need to be tried for different prions, using not only different selection conditions but also imposing stronger or weaker selection conditions of a particular kind (say, for instance, different concentrations of an antifungal-like fluconazole in case of the [MOD^+] prion). For a given prion, different variants ('strains') of that prion could be evaluated, analogous to testing different alleles of a gene, since these variants can differ in phenotypic effects as well as stability and persistence. Such studies would further need to be compared in different genetic backgrounds (yeast isolates). It would also be interesting to carry out selection experiments under constant versus fluctuating conditions, and to track whether prion-associated phenotypes become genetically fixed under either of these regimes (the prediction would be that stable conditions would lead to genetic fixation while fluctuating conditions would be less likely to do so). Besides comparing evolution of prion+ with prion− cultures, cultures which are initially prion-less could be used with backgrounds in which prions can arise *de novo* at reasonable frequencies ([PIN^+] backgrounds), or cannot do so ([pin^-] backgrounds). In addition to the variety of regimes under which such experiments can (need to) be done, direct competition experiments with mixed cultures of prion+ and prion– cells might also be very informative using reporters (a variety of which have already been developed) capable of distinguishing prion-containing cells from prion-less ones.

Apart from lab evolution experiments, a deeper understanding of the mechanisms or determinants of prion switching rates, and whether these might be under environmentally or physiologically coupled cellular control will be not only interesting per se, but would also be valuable in trying to model the behaviour and dynamics of prions in relation to survival and adaptation. Further, it will be useful to investigate whether, or under what conditions, different prions might engender genetic loads on cells (for instance by driving to fixation genetic alleles that might

be adaptive in the short term but deleterious over longer periods).

Lastly, the prevalence of prions in fungi begs the question whether such protein-based epigenetic inheritance (besides the PrP^{Sc} prion of spongiform encephalitis infamy) might also operate in higher eukaryotes like us. So far, the evidence is scanty. Hsp104, central to prion inheritance in fungi, is absent in higher eukaryotes. One example of a prion-like protein that may have a functional role is that of the neuronally expressed CPEB protein (Si *et al.* 2003). March *et al.* (2016) have discussed the presence of possible prion-forming domains in many proteins of higher eukaryotes. Only time and further research will reveal whether any of these do indeed act as prions, and whether some of them might have effects other than triggering neurodegeneration. Given the capacity of living systems to constantly surprise us, we would do well to keep an open mind on this question.

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