Chapter 16

Epigenetic Inheritance and Evolutionary Adaptation

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CONTENTS

Abstract

Although epigenetic inheritance has been recognized to be crucial to maintain different cellular states during development, it is still unclear whether and how often epigenetic marks can be important in adaptation. As epigenetic inheritance encapsulates a wide range of phenomena, we first briefly describing the mechanisms behind the heritable potential of (1) metabolic steady-state systems, (2) cellular structural elements and (3) chromatin marks including DNA methylation. Next, we discuss the experimental evidences for the transmission of chromatin marks through meiosis. Although these results provide a clear mechanistic

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basis for heritable epigenetic variation, an important possible objection is that they might not be stably transmitted through meiosis (and hence not between generations). Moreover, in many cases, epigenetic marks are not inherited in a Mendelian fashion; they are either transmitted to too many progeny, in which case they can also be deleterious, or to too few, in which case even if advantageous they will often be lost. This suggests that under sexual reproduction — possibly associated with cell fusion — epigenetic inheritance is unlikely to play a fair Mendelian game, which is a prerequisite for adaptive evolution.

16.1 Introduction

There is a growing recognition that there is more to heredity than DNA. That this might be so has come to prominence through the study of how it is that the genetically identical cells of an embryo come to have, and stably maintain, different fates (Holliday, 1987). This non-DNA-based heredity, called epigenetic inheritance, although initially a vague concept has gained a secure mechanistic basis and is now crucial to understand development. In principle, the same sorts of epigenetic modifications if transferable between organism generations could be a source of heritable variation (Jablonka and Lamb, 1989, 1995; Maynard Smith, 1990). It is tempting therefore to speculate that there is more to adaptation than the fixation of point mutations, deletions or insertions (Jablonka and Lamb, 1995). But is this speculation reasonable? We argue that although a mechanistic basis removes epigenetic inheritance from the realms of vague speculation, it is still unclear whether and how often epigenetic marks can be important in adaptation, even though they may contribute to heritable variation.

It should be recognized that epigenetic inheritance encapsulates a broad range of phenomena. Jablonka and Lamb (1995, 1998) recognized three main types of systems that can contribute to cellular inheritance: (1) steady-state systems, (2) structural inheritance and (3) chromatin marking systems. We first briefly describe the mechanisms and evolutionary potential of the first two systems. Next we describe the mechanisms behind the inheritance of chromatin marks and how from this basis we can understand the nature of heritable epimutations (Kermicle, 1978). An important possible objection to the idea that epimutations might be important is that they might not be stably transmitted through meiosis (and hence not between generations). We therefore review what is known about this process. Finally, we point to what we believe is the more serious objection, this being not that epimutations are not transferable between sexual generations but rather that the rate at which it occurs must exist in a very particular window (neither too high nor too low) and that only some of the known cases fulfil this requirement.

16.2 Steady-State Systems

It has long been noted that some regulatory and metabolic patterns might have hereditary potentials (Novick and Weiner, 1957). A simple example is autoregulatory genes, which regulate their own transcription by positive feedback. Once turned on, the gene activates its own transcription. If cell division is more or less equal and the concentration of the gene product is high enough in the cytoplasm, the daughter cells might inherit the active state of the gene. In multicellular organisms, many positive gene regulatory feedbacks have been found (Serfling, 1989), supporting the notion that these loops have an important role in maintaining active gene states during somatic development.

Owing to recent advances in genome projects, we now have a detailed knowledge of regulatory networks in *E. coli* (Shen-Orr et al., 2002) and baker's yeast (Guelzim et al., 2002). The networks of these organisms also contain several positive regulatory feedbacks at either the transcriptional or posttranscriptional level. The mechanism also works at the

posttranslational level: it has been shown that some enzymes are needed for their own assembly. For example, Hsp60p (Cheng et al., 1990) and Yah1p proteins (Lange et al., 2000) are found active in the mitochondrial matrix in yeast and are needed to produce additional active enzymes. The loss of proper localization of these enzymes is therefore a potentially irreversible change.

There is also evidence that positive regulatory feedback loops contribute to heritable clonal variation in the expression of the lac operon (Novick and Weiner, 1957). When *E. coli* is cultured under low concentrations of the galactose, two cell types can be distinguished, those with fully active or fully repressed lac operons, and these cell states can be stably inherited for hundreds of generations. The difference is due to the initial stochastic fluctuation in the intracellular concentration of the transport inducer of galactose (permease). This gene is part of the lac operon, and therefore its own induction also depends on the intracellular concentration of galactose. Hence, once the permease is above a critical concentration because of stochastic events, it will increase the concentration of galactose in the cell and hence activate its own transcription. Remarkably, after removal of the nutrient, the cell still maintains the activated state of the lac operon for some generations. It is tempting to speculate that this mechanism is an adaptive response to short spatial or temporal fluctuations in nutrient concentration. It is possible that cells cannot find the nutrient in a given microenvironment even when the extracellular concentration of galactose is still relatively high. In this case, it would be unwise to shut down the operon immediately; instead, it would be better to wait for some generations and ensure that the nutrient is no longer present. Hence, as the cells cannot judge the outer concentration of the nutrient at 100% accuracy, some lag in response to changing environmental conditions might sometimes be favorable.

The inheritance of phenotypic state in this example critically depends on the assumption that the permease is part of the operon and is therefore induced by lactose. Therefore, the comparison of the lac operon structure with the ecological conditions in different bacterial species might shed some light on the possible advantages of this feedback loop.

16.3 Structural Inheritance

The common feature of the following examples is that preexisting cell structures can be used as templates for the assembly of new structures (Jablonka and Lamb, 1995). We consider three examples: cortical inheritance, genetic membranes and prions.

16.3.1 Inheritance of Cell Surface Structure

Some of the earliest examples of nongenetic heredity come from ciliates. It has been known since the early 1960s that the large-scale structure of cortical surface of ciliates shows nongenetic inheritance (Jablonka and Lamb, 1995). The cell surface of these protozoa is covered by thousands of cilia arranged in longitudinal rows and the same orientation. In a series of classical experiments on ciliates, Sonneborn showed that variants of structural organization of the cell surface are stably inherited (Tamm et al., 1975; see also Frankel, 1989). The interpretation for cortical inheritance is that the old kinetid (cilia plus associated structures) serves as a molecular scaffold on which the new one is built. When the orientation of the kinetid is inverted, the new kinetids show the same orientation. Although the example is suggestive, it is unclear whether any of the structural variants could confer advantage over the wild-type organization. Nevertheless, it is likely that structural inheritance associated with cytoskeleton structure is more frequent than what was previously thought. The positional inheritance of the flagellum in trypanosomes is another example of such a phenomenon. It has recently been shown that the old flagellum directs the morphogenesis

and position of the new flagellum in relation to the cell body, and consequently of the internal cytoskeleton (Moreira-Leite et al., 2001). A similar phenomenon has been observed in maintaining the bipolar pattern of budding in yeast (Jablonka and Lamb, 1995).

16.3.2 Genetic Membranes

There are also claims that membranes have some hereditary capabilities (Cavalier-Smith, 2000, 2001; Szathmary, 2000). It has been recognized that mitochondrial membranes are autocatalytic for the incorporation of some proteins. The majority of mitochondrial proteins are encoded by the nucleus, and hence they must somehow be imported to the mitochondria. This process is facilitated by import proteins, which are themselves encoded by the nucleus. However, these import proteins must also be imported, leading to a positive feedback loop for the incorporation of these proteins. Although the idea of membrane inheritance is purely theoretical at this stage, some new experimental results are consistent with this notion. At least two mitochondrial matrix proteins — Hsp60p and Yah1p — are known to be essential for their own assembly. Moreover, some yeast strains seem to be the result of a heritable structural alteration in mitochondria and are not simply genetic mutations in the mitochondria (Lockshon, 2002). These strains are known to be defective in certain steps of leucine biosynthesis that involve transport across mitochondrial membranes.

16.3.3 Prions

Prions provide another example of structural inheritance (Prusiner, 1998). Prions are generally known as infectious agents widely implicated in a variety of mammalian neurodegenerative diseases, e.g., bovine spongiform encephalopathy (BSE), scrapie of sheep and Creutzfeldt–Jakob disease (CJD). The infectious nature of these proteins comes from the ability of the prion protein to catalyze its own propagation. These proteins can have at least two stable conformations: normal and prion. The prion form might rarely arise as a spontaneous misfolding event, possibly facilitated by translational errors. The prion form induces the normal protein to adopt the altered prion conformation, probably leading to the accumulation of amyloid protein aggregates.

Prions are also found in baker's yeast and in the fungus *Podospora* (Wickner et al., 1999). In contrast to mammalian prions, some evidences suggest that the prion form is not fatal to the organism, although the exact biological function is generally unknown. In *Podospora*, the prion form influences cell fusion incompatibility. In the case of yeast, at least three different proteins with prion forms are known (Bradley et al., 2002): (1) [Psi$^+$], the prion form of a translational termination factor protein (Sup35); (2) [URE3], the prion form of a regulator of nitrogen metabolism (Ure2); and (3) [Pin$^+$], the prion form of Rnq1. Remarkably, in all cases, the prion forms can be stably propagated through asexual cell division for hundreds of generations: the frequency of loss is less than 0.8%. In contrast, they are more frequently eliminated during meiosis (2%). Notably, a heat-shock protein (HSP104) is very highly expressed in sporulating cultures (Sanchez et al., 1992). As overexpression of this protein is known to eliminate [PSI$^+$], it is suggested that overexpression might be an evolved response against the rapid spread of prions under sexual reproduction.

Although different prions, e.g., [PSI$^+$] and [Pin$^+$] or [PSI$^+$] and [URE3], can be maintained together in the same cell without much interference, the different prions influence the *de novo* appearance of each other both positively and negatively. [PSI$^+$] and [Pin$^+$] catalyze the formation of each other, and hence their relationship can be considered mutualistic. In contrast, although [PSI$^+$] facilitates the *de novo* appearance of [URE3], [URE3] inhibits [PSI$^+$].

Even more remarkably, numerous distinct strains of the [PSI+] prion form exist (Bradley et al., 2002). These strains are likely to be alternative prion conformations of the same protein (Sup35) and differ in their mitotic stabilities (frequency of [PSI+] loss) and translational termination efficiencies. In contrast to the stable maintenance of different prion types, coexistence is not be possible between two variants of the same prion. This makes sense, as the two variants of the same prion compete for the same pool of newly synthesized proteins to reproduce, and the faster growing prion strain eventually outcompetes the slower, less-stable variant.

Why are prions found in yeast? Are they simply epigenetic parasites, or do they confer any advantage to the host? There are some suggestions that the [PSI+] prions facilitate adaptation to stressful environmental conditions by producing new phenotypic variants (True and Lindquist, 2000). True and Lindquist (2000) showed that this prion has a strong and diverse effect on colony growth and morphology, and sometimes confers advantage over isogenic strains that lack the prion form. The prion reduces the fidelity of translation termination process in a heritable manner (Serio et al., 2001). It causes the read-through of stop codons, leading to an abnormally extended peptide. By reducing the fidelity of protein synthesis, the prion generates enhanced variation at the proteomic level (Pal, 2001). Some of the variants produced by the prion might permit survival under fluctuating environmental conditions.

Previous work has also demonstrated that the N-terminal region of the protein (prion determinant) is essential for converting normal proteins to the prion form (Serio et al., 2001). Prion determinant regions have similar very unusual amino acid composition and imperfect oligopeptide repeats, suggesting that these properties might underlie prion-based inheritance. Similar domains are widely found in eukaryotes (Michelitsch and Weissman, 2000), suggesting that prions might occur more frequently than generally thought. Deletion of this region does not have any harmful effect on colony growth under normal conditions. Remarkably, the region is well conserved across species (Santoso et al., 2000) and is under stabilizing selection (Jensen et al., 2001). However, this is not proof that this region is maintained to provide the prion conformation: there is some evidence that the prion determinant interacts with cytoskeletal proteins (Bailleul et al., 1999).

16.4 Inheritance of Chromatin Marks: Some Mechanistical Considerations

The most prominent examples of epigenetic inheritance are based on the transmission of specific patterns of chromatin structure, or chromatin marks (Jablonka and Lamb, 1995). Chromatin marks consist of complexes of DNA-binding proteins, RNAs and chemical modifications of the DNA (e.g., DNA methylation). The presence of certain marks does not change the coding property of the gene. Rather, it influences the rate and long-term stability of gene expression. Comparable to the semiconservative replication of DNA, these marks are also frequently inherited after cell division. We first illustrate this mechanism by reference to DNA methylation, which is the best-described type of chromatin mark.

16.4.1 DNA Methylation

In many eukaryotes (Regev et al., 1998), some of the cytosines are methylated. DNA methylation contributes to the control of gene expression, parental imprinting (Nicholls and Knepper, 2001), X-chromosome inactivation in mammals and protection of the genome against selfish DNA (Yoder et al., 1997). Methylation usually inhibits transcription initiation, although it is also known that DNA methylation affects transcript elongation in fungi (Martienssen and Colot, 2001). In numerous cases, the DNA methylation pattern of given

genomic regions is faithfully transmitted to daughter cells after cell division. How is this achieved?

According to the most prominent model, methylation patterns can be inherited if cytosines of palindromic sequences (such as CpG or CNG triplets, where N denotes any of the four base pairs) are involved. Because of complementary base pairing, the daughter strand also has the same sequence. Following DNA replication, the parental strand is methylated whereas the daughter strand is not. An enzyme complex including methyltransferase recognizes the hemimethylated state and appropriately methylates the daughter strand. By this mechanism, a pattern of methylated and nonmethylated cytosines is copied, leading to inheritance of silenced or expressed states.

Although many experiments support this model, it must be emphasized that it cannot provide the whole picture. Most importantly, cytosine methylation is not confined to CpG or other symmetrical sequences in plants and fungi (Martienssen and Colot, 2001). It is also known that after treating with demethylating agents, the genomic level of DNA methylation is drastically reduced. However, following removal of the drug, methylation level slowly recovers (Bird, 2002). This result can hardly be explained without assuming some *de novo* methylation process. Indeed, in mammals, an extensive demethylation process occurs in the primordial germ-cell stage and during early development (Yoder et al., 1997). We also have good evidence for the enzymes with some *de novo* activity and others responsible for removing methyl groups (Bird, 2002). It seems that methylation patterns are maintained at a genomic domain level, even if some of the constituting cytosines do not reside at symmetrical sites (Bird, 2002).

There is also hope for a better understanding how DNA methylation affects gene expression level. In an elegant study, Amedeo et al. (2002) identified a gene in *Arabidopsis* whose product is required to maintain transcriptional gene silencing. Mutation of this gene leads to reactivation of several genes, even though these genes remain heavily methylated. Possibly, this gene is involved downstream of methylation in epigenetic regulation.

16.4.2 Chromatin Remodeling

Inheritance of silenced epigenetic state can occur without DNA methylation. DNA methylation is completely absent in numerous model organisms, including *C. elegans* and fission yeast (Regev et al., 1998), and it is also rare in *Drosophia* (Lyko, 2001). Nevertheless, these organisms provide examples of mitotic and meiotic transmission of epigenetic silencing by DNA-protein and protein–protein interactions. The proposed mechanism is remarkably similar to the classic model of DNA methylation, and it is largely inspired by results on the interaction of polycomb-group response elements in *Drosophila* (Lyko and Paro, 1999). Assume that certain chromatin proteins have an ability to bind to certain DNA regions and also to each other. If the association of these chromatin proteins is facilitated by cooperative interactions, then more the proteins found on a certain genomic region, higher the possibility that a new protein can be attached. After replication, the semibound sites of the new DNA molecules could be preferential sites for the assembly of new complexes on the daughter strand.

16.4.3 Histone Acetylation

Histone acetylation is another heritable modification of chromatin structure and like DNA methylation is also involved in such processes as genomic imprinting (Turner, 2000). Acetylation reduces the affinity of the H4 histone protein to DNA, leading to relaxed chromatin structure and higher transcription rate. In contrast, deacetylation of H4 is associated with highly condensed DNA, with low or no transcription. Several mechanisms have been proposed to explain how the acetylation pattern can be transmitted after DNA replication.

One possibility is that the enzymes responsible for acetylation process form part of a complex that remains associated with its target DNA throughout the cell cycle. Alternatively, the enzyme responsible for histone acetylation affects genomic regions with some DNA methylation. Indeed, it is known that the histone deacetylase enzyme and methyltransferase interact with each other (Fuks et al., 2000).

16.4.4 RNA-Mediated Gene Silencing

It has recently become obvious that in many organisms, including fungi, higher plants and animals, small RNAs derived from cleavage of double-stranded RNA are involved in post-transcriptional gene silencing (Kooter et al., 1999; Matzke et al., 2001). Although the details are somewhat obscure, these diverse silencing mechanisms have some common features, indicating an ancient origin. It is claimed that RNA silencing evolved to counter the spread of viruses and transposable elements, many of which produce double-stranded RNAs during their replication. It has also been suggested that host defense mechanisms provided a raw material for the evolution of new regulatory mechanisms for host genes required during development. Many genes are known to contain TE insertions, which might have imposed changes in the regulatory control of the genes.

There is also evidence that small RNAs can guide *de novo* methylation of homologous DNA sequences. Another interesting feature of RNA-mediated gene silencing is that they produce mobile signals (small RNAs) that can potentially induce silencing in cells distant from the origin. These results open an intriguing possibility that RNA-mediated gene silencing can provide a feedback from somatic to germ cells (E. Jablonka, personal communication). Small RNAs derived from somatic cells might move to germ cells and induce *de novo* methylation.

16.4.5 Fidelity of Transmission and Epimutations

Whatever the exact mechanism by which DNA methylation pattern and chromatin marks are transmitted, it is clear that the copying process has limited fidelity. The infidelity of replication of methylation patterns has the capacity to generate heritable phenotypic diversity among genetically identical cells. In almost all somatic cells, illegitimate transcripts occur as a result of spontaneous reactivation (Chelly et al., 1989; McAdams and Arkin, 1999), and this process is especially pronounced during ageing (Brown and Rastan, 1988; Catania and Fairweather, 1991). There is also increasing evidence that methylation changes are involved in cancer initiation (Jones and Laird, 1999; Ohlsson et al., 1999), although it is less clear whether these changes are the result of somatic mutations or they precede it. Kermicle (1978) has termed randomly produced modifications of epigenetic silencing *epimutations* and the possible variants at a given locus *epialleles*.

16.5 Inheritance of Chromatin Marks through Meiosis

It has long been known that the cortical surface structure in asexual ciliates and metabolic states in *E. coli* show nongenetic inheritance (see previously). In these cases, genetically identical populations show clonal heritable variation in these traits. However, this variation may only be maintained because these organisms are unicellular and lack specialized gametes. It has long been argued that in multicellular organisms developing from a single cell, resetting epigenetic information during gametogenesis is necessary to restore totipotency. Does this also imply that *all* epigenetic marks are erased during gametogenesis? Were this so, then clearly, epimutation cannot be important to the process of adaptation. We briefly review some of the best examples for the inheritance of chromatin marks through meiosis to

establish that this objection is not terminal for the speculation that epimutations are involved in adaptation.

It is important to emphasize that we consider only epigenetic traits that are inherited for numerous organism generations; therefore, we do not discuss many other epigenetic phenomena, such as genetic imprinting. Imprints are established and erased every generation in a parental-specific manner, and hence these marks cannot be inherited in the long term and are instead under genetic (DNA-based) control and are better regarded simply as the mechanism by which the imprinting genes exercise their effects.

Consider first a unicellular organism in which chromatin marks are inherited through mitosis and meiosis. In the fission yeast *Schizosaccharomyces pombe*, the two mating cell types (plus and minus) switch efficiently by interchanging alleles at the mating type locus (mat1). This process occurs by directed gene conversion of the information located at the silent mat2 and mat3 loci. These loci are located 11 kb away from the active loci. When a reporter gene is inserted within the regions between the two silent loci, expression from this gene is also greatly reduced (Grewal and Klar, 1996; Grewal, 2000). The authors also identified *cis*-acting regions and *trans*-acting factors responsible for the inheritance of silent states. A partial deletion of these regions results in variegated expression of the inserted gene. More precisely, some of the cell lines with the genetic modification express the transgene whereas others do not. Even more remarkably, the silent or active state of the reporter gene is stably inherited through mitosis and meiosis. The rate of switching between the two states is relatively low rate (once in every 30 to 100 generations).

Heritable epigenetic silencing is observed not only at the mating type region in fission yeast but also at centromeric regions (Nonaka et al., 2002). Remarkably, in both cases, a chromodomain protein (swi6) is involved, suggesting a connection between silencing at these loci.

Epigenetic inheritance through meiosis is not restricted to unicellular organisms. For example, Kakutani and colleagues investigated an *Arabidopsis* mutant (ddm1) defective in maintaining methylation patterns (Kakutani et al., 1999). This leads to a reduced methylation level along the genome and consequently to numerous developmental changes. These changes are stably inherited even when segregated from the mutant genetic background. Similar examples of heritable epigenetic variation in plants were described for laboratory strains, as in the case of transposons of maize (Martienssen and Baron, 1994) and some repeated transgenes of tobacco (Park et al., 1996). In these examples, mutations causing genome-wide demethylation unleashed numerous heritable developmental abnormalities, even if the mutant gene is no longer present. Stable inheritance of methylation changes for numerous generations enables the identification of many controlled genes by conventional linkage analysis and cloning (Habu et al., 2001). This approach appeared to be especially fruitful: heritable epigenetic silencing associated with locus-specific DNA methylation changes have been documented for numerous genes involved in plant developments, including superman (Jacobsen and Meyerowitz, 1997), agamous (Jacobsen et al., 2000) and the flowering locus WA (Soppe et al., 2000).

There is also evidence for the occurrence of epigenetic variation in nature (Cubas et al., 1999). More than 250 years ago, Linneaus described natural variation of flower symmetry in *Linaria vulgaris*. Mostly, the flower of this plant is bilaterial, but radial flowers also occur. It has recently been revealed that epigenetic changes are responsible for this natural variation. The authors have investigated a gene (Lcyc) that controls floral dorsoventral symmetry in this and many other plant species. They failed to find specific genetic changes responsible for the variation. Rather, they found that the gene is extensively methylated and silent in radial variants. The epimutation is transmitted to future plant generations, although much less efficiently than genetic mutations: it was reported that demethylation during somatic development did sometimes occur, reverting to flowers with radial symmetry.

It has long been argued that because of early separation of germline and soma, meiotically heritable epigenetic variation cannot occur in most animal species. On a similar vein, others argued that epigenetic modifications that suppress gene activity in mammals are cleared in the mammalian germline, restoring totipotency of the genome. However, there is direct evidence for the transmission of chromatin marks through meiosis in *Drosophila* (Cavalli and Paro, 1998) and mammals (Morgan et al., 1999; Sutherland et al., 2000).

For example, in an elegant study, Cavalli and Paro (1998) demonstrated that a DNA regulatory motif (Fab-7) confers heritable states of expression and repression during somatic cell divisions. This motif is also known to be located in the homeotic gene cluster, and it maintains the expression state of developmental genes by being the target of protein complexes that organize heritable chromatin structures. Strikingly, the derepressed and depressed states of reporter genes are transmitted to the progeny through the female germline.

In another study, Morgan et al. (1999) described epigenetic inheritance at the agouti gene of mice. Insertion of a retrotransposon upstream of the gene results in ectopic expression of its gene product, with characteristic phenotypic changes (e.g., yellow skin color and obesity). The phenotype also shows variable expression in isogenic mice and it is maternally heritable. Hence, isogenic strains (all containing the inserted retrotransposon) differ only in the expression of the agouti locus, and these changes are also transmitted to the progeny. The possibility of a simple maternal effect was excluded. These results prompted the authors to suggest that mobile genetic elements can produce heritable phenotypic variation (Whitelaw and Martin, 2001). In a similar vein, Sutherland et al. (2000) have revealed that silent state of the transgene in mammals is inherited for multiple generations irrespective of the sex of the parent, implying maintenance of the epigenetic state through meiosis. Furthermore, silencing is transcriptional and correlates with methylation of the transgene as well as an inaccessible chromatin structure; these changes are reversed when expression is reactivated. For other examples of epigenetic inheritance in mammals (including human), see Rakyan et al. (2001).

Although these studies suggest that epigenetic inheritance can occur in organisms with early separation of germline and soma, there are many more examples of the phenomenon in plants. This might simply reflect the advance of certain genetic techniques in plants (such as forward genetics), but differences in the role of DNA methylation might also have some role (Habu et al., 2001). For example, in contrast to plants and fungi, mutants defective in DNA methylation are generally inviable in mammals (Li et al., 1992). Hence, heritable epigenetic variants that could cosegregate with mutants can arise much less frequently, as these mutants have more serious consequences on fitness.

It is important to emphasize that transgenerational epigenetic inheritance is not restricted to organisms with extensive DNA methylation. In *Drosophila*, DNA methylation is barely detectable (Lyko, 2001), and it is likely to be completely absent in fission yeast. Nevertheless, some of the most detailed studies on epigenetic inheritance are from these organisms. Hence, although DNA methylation can have an important contribution, it is neither necessary nor sufficient to maintain expression states.

Nevertheless, it is still very likely that there is a common mechanism behind epigenetic inheritance; for example, the chromatin proteins responsible for heritable gene silencing in fission yeast, *Drosophila* and plants are related to each other (Klar, 1998; Habu et al., 2001).

16.5.1 Is Epigenetic Inheritance a Specific Property of Certain Genomic Regions?

Although the previous examples indicate that epigenetic marks can be transmitted between generations, an important caveat is that all regions of the genome need not be equally likely to permit such inheritance, i.e., it is conceivable that epigenetic inheritance through meiosis is restricted to certain genomic regions, which are somehow safeguarded from the erasure of chromatin marks. For example, in contrast to housekeeping genes, methylation patterns

of certain retrotransposons (e.g., Alu) remain relatively unchanged in the female genome during mammalian development (Yoder et al., 1997). Some other facts point in the same direction. First, epigenetic inheritance of certain traits is often associated with transposable elements (Fedoroff et al., 1995; Whitelaw and Martin, 2001). Second, there is also evidence that heritable gene silencing of the Mu transposon and paramutation in maize are mechanistically linked (Lisch and Carey). Hence, housekeeping genes that happen to be close to regions with many transposable elements might have a relatively high chance to show epigenetic inheritance.

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This regionality potentially allows us to reconcile the finding that radical changes in chromatin structure and methylation patterns occur in mammalian genomes during gametogenesis and early embryogenesis, while there is evidence in mice that certain epigenetic marks can be transmitted to the offspring.

16.6 Evolutionary Potential

Is heritable epigenetic variation merely an aberrant manifestation of developmental processes, as often claimed, or can it also have important roles during evolution? Jablonka and Lamb (1995) argue that epigenetic inheritance provides heritable phenotypic variation, which can be exploited during adaptation to new environmental conditions. Although theoretical models that assume asexuality support their verbal argument (Lachmann and Jablonka, 1996; Pal and Miklos, 1999), the case for adaptation in sexuals must be different.

Consider a simple model (Keller, 1995). Assume that an epimutation has arisen that is neutral. Initially, it will be present in heterozygotes only. What proportion of the progeny of the individual with the epimutation will also have the epimutation? Were the epimutation a point mutation of another DNA-based mutation then, owing to Mendelian inheritance, the answer would be 1/2. Therefore, there would be no deterministic force acting to reduce the allele's frequency, because it must be assumed that the bearer of the mutation has the average number of progeny (i.e., 2). But with epimutations Mendelian inheritance need not be supposed. Many are removed when passaged through meiosis. If k is the proportion of progeny of a heterozygote bearing the epimutation, then $k < 1/2$ might well be true; alternatively, in some instances, $k > 1/2$ might be true. In either case, such epimutations are unlikely to provide raw material for adaptation. In the former case ($k < 1/2$), an advantageous mutation can easily be deterministically lost owing to the failure to be transmitted. If the beneficial effect of the allele increases fitness by s in the heterozygotes, then $s > (1 - 2k)/2k$ must hold for the selection effects to permit spread. Much as with Jeffries objection to Darwinism under blending inheritance, the advantageous mutation must be very advantageous to counteract the transmission system. In the latter case ($k > 1/2$), a deleterious epimutation (with fitness effects $1 - s$) can spread so long as $2k(1 - s) > 1$; that is, the spread need not be associated with advantageous alleles. This does not mean that all such traits will be deleterious. However, if most epialleles are deleterious, then if $k > 1/2$, epimutations are expected to act against the process of adaptation. Only when $k = 1/2$ does s — and s alone — matter. In a finite population, however, there is probably a domain around $k = 1/2$ for which an epimutation can be regarded as effectively Mendelian (much as with s, $s < 1/2$ Ne ensures a mutation to be effectively neutral, where Ne denotes the effective population size).

However, such considerations add an extra wrinkle to the analysis. It must be supposed that for a small Ne, the zone of effective Mendelian transmission is the largest. But with a low Ne, the zone for s being effectively neutral is also larger and hence the zone for adaptation also shrinks. Clearly, one needs to ask what k is for epimutations.

16.6.1 Why k<1/2 Might Often be the Case

For an epimutation to be transmitted with $k = 1/2$, it must not be lost in cells that are not dividing, or when cells are dividing or through meiosis. What is the fidelity of the molecular mechanisms responsible to maintain epigenetic marks? Methylated GCs can spontaneously arise and be lost, partly as a result of the imperfect copying process and because there is evidence of *de novo* activity of certain enzymes (Bird, 2002). Although the replication accuracy of methylation patterns *in vivo* is unknown, *in vitro* studies suggest that it is 95 to 99% per site per cell generation (Holliday, 1987). Obviously, the fidelity of this process is lower than that of DNA replication. However, it is unclear what fraction of the methylated CGs is necessary to maintain the silenced state of a given gene. There are some claims that even if numerous methylated GCs are lost around the genic regions, the silenced state of the gene can remain.

For these reasons, it is important to ask about the fidelity by which the silenced state of a given gene remains through cell divisions. Works on mammalian and plant cell cultures have revealed a remarkably stable inheritance of epigenetic variants. For example, in tobacco and maize cell cultures, the rate of such changes is ca. 10^{-4} to 10^{-6}, respectively, although lower fidelity has also been observed in other cases (Jablonka and Lamb, 1995).

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Most importantly, what is the transmission fidelity of epigenetic marks through organismal generations? Unfortunately, there is almost no systematic approach to investigate this question. Mostly, the fates of the epigenetic variants were followed only for one or two generations. Therefore, we have only very crude estimates of the proportion of progeny to which it can be transmitted. However, there exists the strong generality that marks might be transmitted fairly well through female meiosis but not through male meiosis (Cavalli and Paro, 1998; Morgan et al., 1999). This suggests that often k Å 1/4 must be the case. (Half the time the epimutation will be in a male.) In some cases, the rate is lower still. For example, inheritance of the expression state at the reporter gene in *Drosophila* was possible only through maternal germline, and only 30% of the descendants down the maternal line inheriting the new trait (Cavalli and Paro, 1998) suggests k Å 0.15, clearly a very low fraction.

However, in other cases, a highly reliable transmission was observed. For example, changes in the protein complexes bound to the Y chromosome in *Drosophila* have affected gene silencing in nearby genomic regions and these changes have been faithfully transmitted for 11 generations (Dorn et al., 1993). Silencing of the mating type locus in fission yeast provides another example of a stable inheritance of chromatin marks (Grewal and Klar, 1996).

Furthermore, as pointed out by Maynard Smith and Szathmáry (1995), for epigenetic inheritance to be important in multicellular organisms, it is not enough that chromatin marks are stably inherited through the germline. It is also important that they have reproducible phenotypic consequences in somatic cells. Hence, if a silent epigenetic state is transmitted through the germline, the silent state is expected to be maintained in somatic cells in a similar manner. In the examples of the previous section, this seems to be generally the case. However, there are also examples of partial reactivation of the silenced epigenetic state in somatic cell lineages, resulting in a variegated phenotype (Rakyan et al., 2001). For example, in the agouti loci in mice, there is only partial inheritance of the phenotypes. The descendants generally show a full spectrum of phenotypes (showing more or less somatic expression of the gene), with increased frequency of those that are similar to the mother.

16.6.2 Why k > 1/2 Might be Found

Epigenetic marks can receive transmission at rates higher than those found in Mendelian inheritance. Paramutation is an allelic interaction in higher plants that results in segrega-

tionally biased, meiotically heritable changes in expression (Hollick et al., 1997). The best-studied system showing paramutation involves the R locus in maize, which affects pigment intensity in the plant (Chandler et al., 2000). Alleles sensitive to paramutation are called paramutable, and alleles that initiate paramutation are paramutagenic. Under heterozygotic conditions when a paramutable allele meets a paramutagenic one, the expression of the paramutable allele is decreased through exposure to the paramutagenic allele. After meiotic segregation, the former paramutable allele retains its lowered expression state and itself becomes paramutagenic. Remarkably, in some cases, the two — paramutable and paramutagenic — alleles are genetically identical to each other and differ only in chromatin structure (Hollick et al., 1997). In contrast to Mendel's first law, the lowly and highly expressed alleles do not segregate unchanged from heterozygotes. The low expression state is heritably transmitted through the formerly highly expressed allele. Hence, irrespective of the selective advantage or disadvantage of the paramutagenic allele, it might deterministically spread in the population because of its overreprentation among the products of meiosis.

A similar problem is likely to arise with structural inheritance and other examples that involve alternative states of the cytoplasm. This problem is elucidated with prions. Under sexual reproduction, prion strains in yeast receive transmission at much higher rates than those found in Mendelian inheritance (Tuite and Lindquist, 1996). Under heterozygotic conditions, when strains differing in the presence of the prion meet, nearly all daughter cells will inherit the prion form. A similar problem is likely to arise with other examples of structural inheritance and steady-state systems.

From an evolutionary viewpoint, paramutation and prions are reminiscent of classical examples of meiotic drive. In the latter case, one of the chromosomes has a more than 50% chance of ending up in functional gametes (Hurst et al., 1996). Biased gene conversion has comparable dynamics, but this process affects only one gene instead of a whole chromosome (Hurst and Werren, 2001). Gene conversion is a nonreciprocal recombination event resulting in the alteration of one allele with the other. It routinely occurs during meiosis and is sometimes biased in one direction, leading to one of the two alleles being overrepresented after segregation. The difference between paramutation and biased gene conversion is that instead of a genetic variant, an expression state is transmitted to the other allele, leading to its increased frequency in gametes of heterozygotes.

From these considerations, it can be concluded that there exists a mechanistic basis for epimutations and that sometimes epimutations are transmissable through meiosis and contribute to standing variation, but these facts alone are not adequate to defend the conjecture that epimutations are an important source of heritable variation that might lead to adaptation. Only a limited subset will have transmission rates within the relatively small window ca. $k = 1/2$ to allow selection — and selection alone — to determine their fate. Although there are some cases for near-Mendelian segregation of the epialleles, these are more likely to be the exception rather than the rule. The opposite is true for most DNA-based mutations.

16.6.3 Range of Heritable Epigenetic Variation

However, suppose that $k = 1/2$. Would then epi- and DNA-based mutations be equally likely to be raw material for adaptation? A further difference between the two is the extent to which they permit variation.

Maynard Smith and Szathmáry (1995) have noticed that heredity systems can have limited and unlimited repertoire of hereditary variants. Genetic systems provide an example of unlimited inheritance, as the possible number of genetic variants is practically unlimited and only a very tiny fraction is actually realized in the population. In contrast, in a system with limited range of possible variants, the same few variants emerge repeatedly. In this

case, the frequency of backward changes is much higher than that of a system with unlimited heredity.

At first sight, epigenetic inheritance seems to be a system with limited hereditary potential. There are some claims that epigenetic silencing is frequently an all-or-none phenomenon; that is, if a single locus is considered, it can have only two heritable states: expressed and silenced. Although it might be generally so, there are some examples with multiple heritable epialleles. For example, in *Arabidopsis*, seven heritable epialleles have been observed at the SUP locus. These epialleles are associated with some differences in excess cytosine methylation within the SUP gene and a decreased level of gene expression (Flavell and O'Dell, 1990). Another study has revealed at least three meiotically heritable epigenetic states of a transposable element in maize, and all these states correspond with slight DNA methylation differences in nearby genomic regions (Fedoroff et al., 1995).

However, it is obvious that when a single locus is considered, the number of epialleles is far less than the practically infinite number of mutational variants a gene can have. A much better figure can be obtained if a combinatorial approach across numerous loci is used (see also (Jablonka and Lamb, 1998). Consider an organism with 10, 000 different loci. If only two possible epialleles per locus are considered (active vs. inactive), the number of possible epigenetic variants is $2^{10,000}$, an enormously large number.

The basic assumption behind this calculation is that silenced and expressed states can be achieved independently for all loci considered. One can argue that only a tiny fraction of these possible variants can be achieved because of various regulatory constraints. It is unlikely that heritable epigenetic variants can arise on a gene-by-gene basis. DNA methylation patterns are likely to be maintained through cell divisions at the genomic domain level instead of at the genic level (Bird, 2002). Therefore, the size of independently methylated domains would be of special importance. Assume that these domains generally include 100 genes. In this case, the possible number of epigenetic variants reduces to $2^{1000/100} = 2^{100}$, still a very large number. Hence, it is safe to conclude that even if the vast space of variation is constrained, epigenetic inheritance could theoretically provide variation on which selection could act. (This number is only a tiny fraction of the possible genetic variants a gene can have.)

16.7 Discussion

Owing to the advances in molecular techniques, there are many good examples of the inheritance of epigenetic traits through both mitosis and meiosis. Although the role of epigenetic inheritance during development is generally accepted, it is much less clear whether heritable epigenetic variation can play a significant role during evolution. We have argued that epimutations are unlikely to be the source of heritable variation that is formed by selection into adaptations: they are either transmitted to too many progeny, in which case they can also be deleterious, or to too few, in which case even if advantageous they will often be lost. This suggests that under sexual reproduction — possibly associated with cell fusion — epigenetic inheritance is unlikely to play a fair Mendelian game, which is a prerequisite for adaptive evolution. Only when transmissibility from a heterozygote (k) is half will selective advantage of the epimutation alone specify the fate of the variant. In all other cases ($k < 1/2$ or $k > 1/2$), the transmission capability of the system interferes with its selective advantage. Hence, unfavorable variants with transmissibility can also spread in the population.

But what if k depends on the environment? It has been known that most chromatin marks are labile and transmission accuracy depends on the environment considered. Therefore, recurrent induction might increase the otherwise low transmissibility (E. Jablonka, personal communication). However, we think that it is not enough if recurrent environmental

changes influence the average value of transmissibility over generations. It is hard to imagine how environmental changes could provide compensation for the otherwise low transmissibility, leading to an average k = _ transmissibility. Consider, for example, in a population a rare epigenetic variant that is generally transmitted to 49% of the offspring. (Note that this is a very slight bias.) After only 35 generations its frequency will be halved, and not even a complete induction (k = 1) that lasts for one generation can restore its original frequency. Assume, however, that induction occurs more often, say after every 10 generations. It easy to see that the original frequency will be restored if and only if the transmission ratio during induction is exactly k_i = 0.612. If k_i > 0.612 the variant will be overrepresented, whereas if k < 0.612 the variant remains underrepresented in the population. It is very hard to see why such a precise induction should occur, unless k is itself a selective trait.

In sum, epigenetic inheritance is expected to have more profound effect under asexual, unicellular conditions. Most theoretical models on epigenetic inheritance consider asexual populations (Pal and Miklos, 1999; Lachmann and Jablonka, 1996). However, we do not consider our argument decisive in this debate. It is possible that our knowledge is biased. After all, most examples of nongenetic inheritance were discovered because of the suspiciously non-Mendelian segregation of the trait considered. Further, we have not considered the possibility that group selection might play a role. We have argued that k > 1/2 will allow easier invasion of both advantageous and deleterious epialleles. As most epialles can be assumed to be deleterious, this process acts to degrade populations. But if we allow for between-group competition (with minimal mixing or sex between the groups), then selection might favor those groups with epimutations (with k > 1/2) over those without, as some groups might, by chance, have more advantageous alleles. A detailed model would be needed to see whether this speculation might be supportable even in theory.

Some facts suggest that fair segregation of chromatin marks during meiosis might occur more often than previously thought. Recently, Riddle and Richards (2002) found significant natural variation in cytosine methylation in *Arabidopsis*, particularly in the nucleolus organizer regions, which constitute ca. 6% of the genome. Their results indicate that besides the differences in *trans*-acting modifier genes and rRNA copy number among the populations, epigenetic inheritance of methylation patterns also contributes to the variation. The authors also noted that gene methylation in F1 hybrids created by reciprocal crosses was intermediate between the two parents (high and low methylation). This could potentially be due to a homogenization of methylation at intermediate value on both parental chromosomes in the hybrids. In this case, the variation would disappear and provide an example of blending inheritance. Alternatively, parental methylation patterns might be preserved and inherited in the hybrids. The latter explanation turned to be true. Some further examples show that hypomethylation of genomic sequences is inherited in a Mendelian manner.

Although it is hard to judge how often Mendelian segregation of an epigenetic trait can occur, it does not follow that epigenetic inheritance cannot have important evolutionary consequences. One need, for example, only considers genomic imprinting. Marks are put on or taken off genes in one germline, effects that are reversed in the germline of the opposite sex. But this is not the independent evolution of a heritable system that runs in parallel to DNA-based polymorphisms. Rather, DNA-based mutations direct the placement and removal of the marks, much as coding sequences determine which amino acids are to be employed.

Further, we should consider some of the consequences of having epigenetic systems. Most notably, transcriptionally active regions are more prone to mutations (Datta and Jinksrobertson, 1995), have higher recombination rates (Gerton et al., 2000) and gene silencing mechanisms influence the invasion possibility of transposable elements and foreign sequences (Yoder et al., 1997). Restriction modification systems in bacteria (a system that

involves DNA methylation) might itself be considered as selfish genetic elements (Kobayashi, 2001).

It is also known that highly expressed genes are under more stringent selection pressure (Pal et al., 2001), possibly to reduce metabolic costs of amino acid biosynthesis (Akashi and Gojobori, 2002). Gene silencing mechanisms could also influence the formation of viable hybrids between related species (Pikaard 2000, 2001). For example, in newly formed allopolyploids of plant species, substantial DNA methylation changes are observed in genes and transposable elements.

We can then be sure that epigenetics is of importance, but it is unclear whether when uncoupled from DNA-based inheritance (i.e., when considered as a system parallel to DNA-based inheritance) it will be of importance in the process of adaptation. Possibly, only under unusual circumstances can epimutations have any lasting role.

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