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Reprints and Reflections

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The Inheritance of Acquired Epigenetic Variations[1]

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Abstract

There is evidence that the functional history of a gene in one generation can influence its expression in the next. In somatic cells, changes in gene activity are frequently associated with changes in the pattern of methylation of the cytosines in DNA; these methylation patterns are stably inherited. Recent work suggests that information about patterns of methylation and other epigenetic states can also be transmitted from parents to offspring. This evidence is the basis of a model for the inheritance of acquired epigenetic variations. According to the model, an environmental stimulus can induce heritable chromatin modifications which are very specific and predictable, and might result in an adaptive response to the stimulus. This type of response probably has most significance for adaptive evolution in organisms such as fungi and plants, which lack distinct segregation of the soma and germ line. However, in all organisms, the accumulation of specific and random chromatin modifications in the germ line may be important in speciation, because these modifications could lead to reproductive isolation between populations. Heritable chromatin variations may also alter the frequency and distribution of classical mutations and meiotic recombination. Therefore, inherited epigenetic changes in the structure of chromatin can influence neo-Darwinian evolution as well as cause a type of "Lamarckian" inheritance.

Introduction

Genetic information resides in the sequence of DNA bases, but more than DNA sequence information is transmitted from one cell generation to the next. What is transmitted is chromatin, a three-dimensional complex of DNA and proteins. Therefore, in addition to the instructions coded in the base sequence of DNA, genes can carry and transmit information embedded in the structure and conformation of chromatin. Such information is epigenetic information[1,2]; it will reflect the developmental and functional history of the genes, and it will be involved in their present and future activity.

The molecular mechanisms underlying heritable changes in chromatin structure and gene function are not fully understood. One factor which is thought to be important is modification of DNA bases, particularly methylation of cytosine.[3,4] It has been shown that patterns of cytosine methylation are stably inherited in somatic cell lineages, and that some changes in gene activity are correlated with changes in methylation patterns.[5,6]

1 Jablonka E, Lamb MJ. The Inheritance of Acquired Epigenetic Variations. *J. theor. Bioi.* 1989;139:69-83. Reprinted with permission.

The identification of altered patterns of cytosine methylation as a possible molecular basis for epigenetic changes in somatic cell lines has led to the suggestion that similar DNA modifications might also occur in the germ line, and thus influence gene expression in the nextgeneration. Holliday[7] has discussed this possibility and explored the origin and consequences of defects in methylation in both somatic and germ line cells. It is clear that epigenetic defects resulting from methylation errors in somatic cells may be important in cell transformation and in ageing. Also, according to Holliday, epigenetic defects in germ line cells could be the reason for the morphological variation found within inbred strains of mice, and for the high incidenceof tumours in the offspring of mutagen-treated parents.

The clearest evidence that epigenetic characters can be transmitted through the germ line comes from studies of genomic imprinting. It has been known for a long time that the expression and transmission of a gene, a whole chromosome, or a whole set of chromosomes sometimes depends on the sex of the parent from which it was inherited (reviewed in Monk[8] and Marx[9]). The genetic material inherited from the male parent must differ in some way from that inherited from the female parent, i.e. the maternal and/or paternal chromatin must be "imprinted"[10] or modified in a way which reflects the sex of the parent and which results in differential expression of the two parental genomes in the offspring.

In the following discussion we shall argue that genomic imprinting is a special case of inherited epigenetic variation. Using a model based on imprinting-like phenomena, we shall explore the evolutionary implications of the evidence that epigenetic variations can be inherited. First, we shall show that it is possible to suggest a plausible model of "Lamarckian" inheritance based on the inheritance of epigenetic variations. According to this model, the relationship between an environmental stimulus and the heritable modification of a gene could be very specific and predictable, and result in an adaptive response to the stimulus. However, we will show that, although this type of Lamarckian inheritance may be quite widespread in plants and in fungi, in the animal kingdom the opportunities for it to lead to adaptive changes are severely limited. Second, we shall show that the accumulation of inherited epigenetic variations may be important in speciation, since it could be involved in establishing reproductive isolation between populations. Finally, we suggest that heritable epigenetic variations may alter the frequency of classical mutations and meiotic recombination, and hence will affect the rate and direction of evolutionary change in another, indirect, way.

The Inheritance of Functional States in Cell Lineages

During embryogenesis, cells undergo a hierarchical series of changes which make them progressively more specialized until they reach a determined state which is usually very stable. Little is known about the developmental cues which provide the positional and temporal information necessaryto initiate the events leading to determined and differentiated states. It is clear, however, that the transmission of these states in cell lineages is frequently independent of the stimulus initially responsible for inducing them. For example, many years ago[11] showed that imaginal disc cells from third instar larvae of *Drosophila* could be cultured in the abdomen of adult femalesfor over a thousand cell generations, and still retain their original determined state.

Although the details of the molecular events underlying heritable changes in the capacity of genes to be transcribed are not known, usually base sequence changes are not involved. Rather, what seems to change is chromatin structure and conformation; it is *the gene's phenotype* which determines its functional state. The structure of actively transcribed genes can differ from that of non-transcribed regions of chromatin in several ways. The features associated with active, or potentially active, genes include increased general sensitivity to DNase-1 and other endonucleases, the presence of HMG (high-mobility group) and other non-histone proteins, modification of histones by ubiquitination, acetylation or phosphorylation, replacement of one histone variant by another, undermethylation of cytosines, and the presence of sites which are hypersensitive to DNaseI1 (reviewed in Reeves[12]; Eissenberg[13]; Gross & Garrard[14]). Exactly how these features are related to gene expression and to each other is not fully understood. At least some of them are stably transmitted through many rounds of cell division. In the case of cytosine methylation, the way in which the pattern of methylation can be passed on to daughter cells is fairly clear. In many eukaryotes, the cytosines which are methylated are found in CpG doublets or CpNpG triplets. Following replication of methylated sites, a methyl transferase recognises the hemimethylated sites and methylates the cytosine of the new strand of the DNA duplex. Since the enzyme catalyses methylation preferentially at sites at which one strand is already methylated, it ensures the heritability of DNA methylation patterns.[7]

Methylation patterns are not the only aspect of the gene's phenotype which can be transmitted to daughter cells. Patterns of DNase-1 hypersensitive sites are also clonally inherited, and plausible models for the way in which these sites and other chromatin modifications can be propagated have been proposed.[15–17]

Environmental Stimuli and the Gene's Phenotype

In recent years, it has been shown that genes whose functional activities change during development undergo progressive changes. The passage from an inactive to an active state seems to be a multistage process, rather than a simple switch. Because of this, terms like "inactive" or "active" genes are no longer satisfactory. "Inactive gene" is not an adequate description because it does not specify how many of the necessary conditions for becoming transcriptionally active have yet to be satisfied. Similarly, the term "activegene" does not unequivocally describe the state of gene activity, because a gene can be either stably or transiently active. Biologists now tend to talk about "determined", "potentially expressible", "inducible", "expressing", "non-expressing", "repressed", "derepressed", etc., states of genes.[18]

In general, it is possible to distinguish two types of changes in response to a stimulus. One is a *visible* response which occurs when an already competent chromatin region switches from a state of transcriptional inactivity to active transcription, or *vice versa*. For example, such an "off-on" response is seen in the heat shock genes which very rapidly become transcriptionally active following exposure to stress conditions.[19] The second type of response is *cryptic:* the competence and stability of the gene are changed, but there is no change in its overt functional state. For example, if the gene was stably inactive, the stimulus does not activate the gene, but makes it "eligible" for activation by the same or another stimulus at a later stage. Alternatively, the stimulus may cause an already inactive gene to become more stably inactive, i.e. less prone to activation. An example of a cryptic response is seen in the adult globin genes during development. At an early developmental stage the genes are inactive and insensitive to DNase-1; they then become DNase-1 sensitive (showing competence for overactivity), although still inactive; at a still later stage they become transcriptionally active.[20,21]

For both visible and cryptic responses, there is evidence that the new epigenetic state of the gene can be propagated during cell division. In this respect epigenetic changes brought about by environmental stimuli resemble DNA sequence changes induced by a mutagenic agent. However, there are important differences. These are summarised in Table1.

As indicated in the Table, mutations are usually random, with a new mutation being unrelated to the stimulus inducingit. In contrast to this, epigenetic modifications can be either random or directed. *Random* modifications are non-specific both with regard to the stimulus which induces them and the gene which is modified. Holliday[7,22] has termed such modifications "epimutations". Typically, the frequency of induction and the frequency of reversion of epimutations are higher than for classical mutations. The reduction in the level of DNA methylation induced by 5-azacytidine leads to this class of epigenetic variation. This chemical does not specifically o rpreferentially induce a change in the methylation of a particular gene, but rather induces a genome-wide reduction in the level of DNA methylation; each gene within a wide category of genes whose expression is regulated by DNA methylation has a certain, and sometimes substantial, probability of being activated.[23] *Directed* epigenetic variations are those produced when the stimulus affects a specific gene in a particular cell type at a particular stage of development. The stimulus has no consistent effect on other genes, or on the same gene in a different tissue or at a different developmental stage. This class of epigenetic modification may be involved in the production of phenocopies, i.e. environmentally induced phenotypic changes which mimic those produced by classical mutations. For example, in *Drosophila*,ether treatment of very early embryos leads to phenocopies of bithorax.[24,25] Although most of the detectable directed modifications which are induced experimentally are likely to be detrimental, there are many examples of directed modifications which are adaptive: the induced modification is advantageous to the cell or to the whole organism because it is part of the normal developmental programme. For example, nuclease hypersensitive sites which are induced in the chicken vitellogenin gene by oestrogen and which are perpetuated in the cell lineage even when the hormone is withdrawn,[26] are directed modifications of chromatin structure which are of adaptive importance.

Epigenetic Variations in the Germ Line and Gametes

In order for an acquired epigenetic variation to be transmitted to descendants, it is necessary for the variation to be present in the germ line and eventually in the gametes. Whether or not this is so will depend on three principal factors: (i) the occurrence and timing of the segregation between the germ line and soma during development; (ii) the frequency of epigenetic variations in germline cells, or in cells that can contribute to the germ line; (iii) the frequency of "reversion", which will depend on the accuracy of the copying system, on the nature of reprogramming and dedifferentiation processes, and on the efficiency of any repair and cell selection mechanisms.

(1) The Segregation of Soma and Germ Line

For both epigenetic variations and classical mutations, the likelihood of transmission is affected by the time at which the "developmental barrier" separating somatic and germ

Table 1. A comparison of induced heritable epigenetic modifications and induced mutations

Property	Induced heritable epigenetic variation	Induced mutation
Type of variation	Does not involve DNA base sequence changes; involves a change in chromatin structure which is likely to affect transcriptional control	Involves a change in DNA base sequence
Frequency of "forward" variation	Very wide range: up to 100% per locus	More limited range: $<10^{-4}$ per locus
Frequency of "backward" variation	Very wide range: up to 100% per locus	More limited range: very low
Locus and tissue specificity	May be highly specific; the probability of a specific change could be 100% for the appropriate gene in the relevant cell type at the appropriate stage of development	The probability of a particular change varies, but is always extremely low
Adaptiveness of the induced response	The modification may have nonrandom, although not necessarily adaptive, biological significance	No connection between the molecular event and its potential biological significance
Transmission through the germ line	Depends on the nature of reprogramming processes and on the efficiency of repair and cell selection	Depends on the efficiency of repair and cell selection processes

line cells is established, and its stability. The earlier in development it occurs, and the more irreversible it is, the smaller the chance that new variations will be present in the germ line. The timing and reversibility of germ line-soma segregation is different for different organisms.[27,28] In organisms without a distinct germ line, germ cells can develop from somatic stem cells. Therefore, if the genes of these "somatic" cells have been epigenetically modified, the modification can, in theory, be transmitted to the germ line and to the organism's descendants. The timing and reversibility of germ line segregation is of particular relevance when considering what we have termed *directed* modifications. A directed modification is, by definition, limited to a particular cell type and particular time, so its transmission to descendants will depend on whether that cell type can contribute to the germ line. In many animal groups, including the mammals, germ line-soma segregation occurs early in development and seems to be irreversible. In such groups, only changes occurring before segregation, or those occurring in the germ line itself, can be passed on to descendants. On the other hand, in plants, fungi and protists, where the developmental barrier does not exist ,directed and adaptive variations affecting somatic stem cell functions could be represented in the germ line fairly frequently, and hence could be of considerable evolutionary significance.

(2) The Frequency of Environmentally Induced Epigenetic Variations in Germline Cells

If the epigenetic change induced in the germ line is a *random* modification, the probability of its occurrence will depend on the potency of the inducing agent. For somatic cells, it is known that 5-azacytidine can cause a 30% reduction in the level of total genomic methylation[29]; silent genes are reactivated in 0.1-30% of survivingcells.[30–33] Unfortunately the frequencyof activation or repression of genes in germline cells by 5-azacytidine or other agents is unknown. For a *directed* modification, an inducer could, but need not, affect 100% of the germline cells. Thus, in an extreme case, the functional state of a particular gene could be alteredin every germline cell.

(3) The Frequency of "Reversion" in Germline Cells

The accuracy of the mechanisms responsible for propagating a gene's phenotype in the germ line is not known. For DNA methylation in somatic cells in culture, the accuracy of copying may be over 99%,[34] although some studies suggest a more substantial drift in methylation patterns,[35,36] There is some evidence that methylation stabilityvaries with the site, with critical sites being more stable.[37] In the germ line, the chances of transmission also depend on the nature of the reprogramming processes which take place during gametogenesis, on the efficiency of mechanisms which repair epigenetic variations which deviate from some recognised "norm", and on detection-elimination mechanisms which preferentially remove cells containing epigenetic variants.

The molecular mechanisms involved in the reprogramming processes which ensure the totipotency of gametes are not fully understood. It seems likely that the changes in chromatin structure such as replacement of proteins,[38] changes in the level of DNA methylation,[39–41] etc. which occur during gametogenesis, particularly during spermatogenesis, play an important role in reprogramming. Since extensive changes in chromatin take place, it could be argued that these changes are likely to erase information from previous stages which is encoded in the structure of chromatin. However, Groudine & Conklin[39] have shown

that this need not be so. They found that in the chicken the chromatin structure of all genes in sperm is different from that seen in somatic cells and spermatogonia, and that the general level of DNA methylation is higher. Nevertheless, the DNA sequences within DNase-1 hypersensitive sites of constitutively expressed genes are marked in sperm by being preferentially undermethylated. Thus the undermethylated regions serve as "footprints" of the past DNase-1 hypersensitive conformation, and could act as "blueprints" for the reconstitution of that conformation in early embryogenesis. Further evidence that blueprints of epigenetic information can be transmitted through the germ line comes from recent work by Silva &White.[42] They showed that in some tissues the pattern of methylation differs at two allelic sites. This variation, which is tissue specific, is inherited in a Mendelian fashion for at least three generations. However, in sperm the methylation pattern of these loci was found to be uniform and sperm-specific. Thus, the variation cannot be transmitted directly, but some blueprint of the methylation patterns must be established during gametogenesis. Silva &White suggested that elements such as DNA binding proteins which segregate with the chromosomes during meiosis may serve as blueprints. Whatever the molecular nature of the blueprint mechanism, this work, like that of Groudine &Conklin, shows clearly that, although epigenetic information is not transmitted unaltered, some characteristics of its structure may be encoded in the chromatin of gametes.

The role of repair and selection processes in eliminating epigenetic variants is unknown. It has been suggested that meiosis plays an important role in repairing both defects in DNA[43,44] and epigenetic defects resulting from loss of methylation.[45] Although it seems highly likely that mechanisms repairing, eliminating or compensating for epigenetic defects do exist, they are at present unknown, so their efficiency cannot be estimated. It is improbable that they are perfect.

Imprinting and Imprinting-like Inheritance of Epigenetic Variations

The evidence outlined above suggests that new, induced, chromatin modifications could persist during cell division in the germ line, could affect the restructuring of chromatin at the reprogramming stage, and could be transmitted as new blueprints to the next generation. However, in order forth is phenomenon to have any evolutionary significance,we have to assume that the chromatin modifications are capable of influencing gene expression during the offspring's development, and that the modifications are transmitted by the offspring to future generations. Evidence showing that epigenetic characteristics are not only transmitted via the gametes, but also influence the expression of genes in the offspring, comes from studies of genomic imprinting. In animal groups as different as mammals and insects, it has been shown that the genetic contributions of male and female parents are not equivalent: the expression and transmission of a gene, a chromosome, or a set of chromosomes sometimes depends on whether it was inherited from the male or the female parent (reviewed by89). The processes which establish the differences between the maternally and paternally derived genetic contributions are known as "imprinting".[10] The most plausible origin of the phenomenon is in the differences in chromatin structure in male and female gametes, which are inevitable consequences of the different ways in which the chromatin is packaged in the sperm and egg.[46] At fertilization, the paternal and maternal chromosome sets have different conformations. The differences are normally erased early in development.[47] However, when they persist for a longer time, phenotypic differences due to the parent-dependent functional state of specific genes or chromosomes become visible, and "parental genomic imprinting" is detected.

It is not clear how widespread genomic imprinting is, but the diversity of groups in which it has been found suggests that it may be quite common. In some diptera and coccids, there is selective elimination or inactivation of the whole haploid set of chromosomes inherited from the male parent.[10,48] In mammals, in almost all somatic cells of marsupials[49,50] and in the extraembryonic tissues of eutherians,[51] the paternally derived X-chromosome is preferentially inactivated. There is also evidence of functional differences between maternally and paternally derived autosomes: mouse embryos which inherit both copies of an autosome from a single parent frequently do not complete development, and if they do, the phenotype of individuals inheriting both autosomes from their mother sometimes differs from that of those inheriting both from their father.[52,53] Further evidence of imprinting in mammals comes from the fact that mouse embryos containing two male pronuclei or two female pronuclei do not develop much beyond the implantation stage.[54,55] What are known as "parental source effects" have long been recognized in *Drosophila*: for example, the extent of position effect variegation, i.e. the mosaic phenotype observed in heterozygotes for some chromosomal rearrangements which place euchromatic genes near heterochromatin, depends on the sex of the parent transmitting the arrangement.[56] Recently, it has been suggested that the effects of imprinting can be seen in a number of human genetic diseases whose expression is influenced by the sex of the parent from which they were inherited.[57–60]

Imprinting must involve parent-dependent chromatin modifications which are capable of influencing gene

expression in the offspring. Differential DNA methylation may be one of the mechanisms involved.[34] Recently, attempts have been made to investigate the molecular nature of imprinting by using transgenic mice. These studies have provided strong evidence that methylation is involved in some cases of imprinting.[58,61,62] In one study it was found that a transgene was expressed only when inherited from the male parent; this male-transmitted transgene was undermethylated compared with the maternally transmitted gene.[62]

In the studies of methylation of transgenes cited above, the pattern of methylation was reversed when the transgene was transmitted through the opposite sex. This is expected if methylation is causally related to imprinting. A somewhat different, but we believe important, observation of an imprinting-like phenomenon has been described by Hadchouel.[63] Their work showed that a transgene which was undermethylated and expressed when paternally transmitted, became *irreversibly* repressed and more highly methylated once it passed through a female; it was inherited in this repressed state for several generations, irrespective of the sex of the parent. The work of Silva &White[42] showed that normal, differentially methylated, genes can behave in the same way as this transgene, i.e. the particular methylation patternof an allele can be stably transmitted, irrespective of parental sex. This evidence showing that epigenetic variations can be inherited is the basis of our model for the inheritance of acquired epigenetic characters. We believe that genomic imprinting, in which the inherited chromatin modifications depend on the sex of the parent, is a special case of a more general phenomenon in which epigenetic events in one generation may, under some circumstances, leave an impression, or *mark,* on the gene, which influences its activity in the next generation. In order to retain the term "imprint" for a heritable chromatin modification which is determined by the sex of the parent of origin, we will use the term "mark" for any chromatin modification which reflects the functional history of the gene or chromosome region in the previous generation.

The Model

The inheritance of an acquired epigenetic variation requires that:

i. An environmental stimulus induces a heritable change in a gene's phenotype.
ii. The stimulus affects either germline cells, or cells which cancontribute to the germ line.
iii. The change in the gene'sphenotype is transmitted to progeny.

We assume that the new phenotype of the gene is not transmitted to the next generation unaltered. Rather, as occurs with imprinting, the chromatin variation is transmitted as a changed mark on the gene in the gametes. This mark can influence the gene's expression in the progeny. However, in order for the new variation to have any evolutionary significance, the progeny must also propagate the new mark, i.e. it must be transmitted through their germ line, be present in their gametes, passed to their offspring, and so on. When this occurs, the new phenotype of the gene, which was originally induced by an environmental stimulus, will be maintained in the descendants whether or not the stimulus is present. In other words, the chromatin variation is stimulus independent - an acquired character has become inherited.

New chromatin variations are always initiated in germline or pre-germline cells, but once they are established in the germ lineage, their effects may be seen either in the germ line itself, or in somatic cells derived from the germ line, or in both. The change in the gene's phenotype will usually result in a change in its time or mode of expression. However, even if there is no change in the activity of the gene as a result of its altered phenotype, the new variation could still be of functional significance since it might make the gene respond to other environmental changes more readily than before modification.

In theory, the stimulus leading to a change in the gene's phenotype could be of limited duration (e.g. a temperature shock), or persist throughout development (e.g. a sustained change in environmental temperature). Furthermore, it could occur just once, or recur in several generations. Although a single stimulus could affect characters in descendants, this is probably unusual, since small changes in a gene's phenotype may fail to establish a novel mark in gametes, or might establish a new mark with neutral effects. For this reason, it seems likely that a number of small changes in chromatin structure have to accumulate before the chromatin is modified sufficiently to create a new mark. This would require a sustained or recurring environmental change. Another reason for thinking that persistent environmental changes may be necessary is that with a single stimulus, even if both copies of the gene in the exposed individual are modified, its offspring may carry only one copy of the new mark. In the germ line this "heterozygous" condition could lead to the gene being detected as deviant and being repaired, or it might even signal the destruction of the cell.

The evolutionary importance of new epigenetic variations is three-fold: (i) chromatin modifications which are transmitted to the progeny may directly affect the tempo and mode of adaptive evolution and speciation; (ii) chromatin modifications in germ cells may have non-random effects on DNA base sequence changes; and (iii) since most inherited epigenetic variations are likely to have deleterious

effects, selection will strengthen systems which minimize the transmission of induced epigenetic variants to the next generation. Such systems include those affecting the timing and stability of germ line-soma segregation, and the repair and elimination processes of the germ line. These were considered earlier in this paper. In the following sections we shall discuss the more direct evolutionary consequences of inherited epigenetic modifications.

Adaptive Evolution and Speciation

Since some epigenetic variations may be directed by the environment, their transmission to the next generation can result in a type of "Lamarckian" inheritance: a hereditary character is transformed by an environmental stimulus in a non-random way. Directed heritable variations will occur in those loci whose activities change in response to changes in the environment. This may affect both the direction and rate of evolution. Loci involved in two types of physiological adaptation are likely to show Lamarckian inheritance: (i) loci controlling global responses such as adaptations to temperature or salinity, since changes in such loci affect almost all cell types; (ii) loci determining germ line-specific adaptations. Since new environments will induce more and different chromatin modifications, inherited epigenetic changes could contribute to rapid adaptation to new niches.

Another consequence of epigenetic inheritance is that a variation can become fixed in a population fairly rapidly, even if it has no selective advantage. This happens because the same directed variation can occur in a number of unrelated individuals when the population encounters a new environment. In the case of a severely detrimental epigenetic variant, if the stimulus is potent and continues for several generations, the result could be population extinction because all individuals would soon carry the new variation.

The effects of a change in the phenotype of a gene will be influenced by the stage in development at which it is expressed. A change in a gene which is expressed early in development is likely to have extensive detrimental effects and usuallyit will be selectively eliminated. Nevertheless, on rare occasions, such a change could cause alterations in the timing of determination and differentiation events which would lead to evolutionary innovations such as various types of heterochrony.[64]

Epigenetic changes in genes which are expressed in the germ line may have particularly important evolutionary consequences, because such genes are usually involved in determining fertility. The accumulation of modifications in these genes may play an important role in speciation. Speciation involves the development of reproductive isolation between populations. Frequently, hybrid sterility and hybrid inviability, which reduce the success of interspecific crosses, are important isolating mechanisms. It is possible, particularly in the early stages of divergence between populations, that such sterility and inviability are the consequences of epigenetic rather than genetic differences between incipient species. This can be illustrated by considering how epigenetic changes could be the basis of reproductive isolation between populations due to (i) sterility of one sex of the hybrids and(ii) sterility of both sexes.

i. According to Haldane's rule, if only one sex of the hybrids between two species is sterile, it is almost always the heterogametic sex.[65] There are two, probably related, reasons for thinking that epigenetic modifications may be involved in this sex difference. The first is that in a least some species, imprinting seems to be particularly marked for sex chromosomes. For example, inactivation of mammalian X-chromosomes is influenced by their parental origin[49–51]; in *Sciara*, the maternal and paternal X-chromosomes behave differently during embryogenesis and during male meiosis.[48] The second reason is that in groups in which the sex chromosomes are heteromorphic, the chromosomes in the heterogametic sex undergo extensive conformational changes during gametogenesis. When these conformational changes are impaired, the result is a reduction in fertility or complete sterility.[66] The marked imprinting of the sex chromosomes and the requirement for precisely regulated conformational changes in these chromosomes during gametogenesis may mean that they are particularly sensitive to changes in their chromatin conformation. If two populations have diverged and accumulated epigenetic variations, then the sex chromosomes may not undergo the required conformational changes during gametogenesis in the heterogametic sex of hybrids. The resulting sterility may be analogous to the reduced fertility found in the heterogametic sex of many species when chromosome aberrations interfere with the conformational changes that sex chromosomes normally undergo during gametogenesis.
ii. In cases where both sexes of the hybrid progeny are sterile, sterility may be due to the accumulation of epigenetic variations in germ line-specific genes. As we have already pointed out, adaptive changes are likely to occur in germ line-specific genes, and these genes probably play an important role in determining fertility. A further reason for the sterility of hybrids may be that chromatin restructuring is defective in the cells of hybrids, and neither autosomes nor sex chromosomes undergo the correct conformational changes.

This could result in germ cells being unable to complete gametogenesis successfully.

There is some evidence suggesting that chromatin structure does indeed differ in closely related strains or species, and is involved in hybrid inviability. Crosses between some strains of the haplodiploid wasp *Mormoniella* produce no, or few, diploid females. Even though sperm enter the eggs, the chromosomes of paternal origin appear disorganized and are not incorporated into the zygote nucleus.[67] Consequently, most of these eggs develop as haploid males rather than diploid females The chromatin from the male parent in these incompatible strains presumably carries different imprints from that of compatible strains. Differences in parental imprinting may also be responsible for the allelic repression found in hybrids from some interspecific crosses in birds and fish.[68,69] Although for some isozymes hybrids express both alleles, for others only one of the parental alleles is expressed. We believe that the accumulation of differences in chromatin structure such as are suggested by these examples may be important in speciation. Isolating mechanisms involving inherited epigenetic differences may explain some of the cases where no significant genetic divergence has been detected between non-interbreeding populations.

Epigenetic Modifications and DNA Base Sequence Changes

The way in which chromatin conformation affects DNA base sequence changes has not been explored in depth. However, the evidence outlined below suggests that the conformation of a chromosome region may influence the frequency of mutation and recombination in that region.

(1) DNA Repair and Mutation

In mammalian cells, the rate of repair of UV-induced damage is higher for actively transcribed genes than for inactive genes. It has been suggested that this may be associated with the more open conformation of DNA in transcriptionally active regions.[70] However, recent work showing that damage to the transcribed strand of DNA is removed more rapidly than that to the non-transcribed strand indicates that chromatin conformation is not the only factor involved[71,72] proposed that malignant transformation following exposure of mouse cells to X-rays involves two steps, one of which is epigenetic. The first step occurs in most cells and involves a change in functional state which is transmitted to daughter cells. This epigenetic change enhances the probability of a second, probably mutational, change which results in transformation. Studies of irradiated mice suggest that similar epigenetic modifications may be induced in germline cells and result in a high incidence of tumors in the progeny.[7]

Recent work by[73] has shown that some mutations in *E. coli* are directed, i.e. in response to the selection agent, the bacteria mutate in ways which enhance their own survival. In all the cases studied, the selection agent also participated in the regulation of gene activity by altering the affinity of regulatory proteins. Therefore, the observed local increase in mutation frequency may have been caused by conformational changes influencing the action of repair enzymes which introduce mutations.

(2) Transposition and dna modification in plants

Some transposable elements in maize undergo reversible changes in activity which have been shown to be correlated with changes in DNA methylation.[74,75] In the cases reported, high levels of DNA methylation were correlated with inability to transpose, whereas low levels were associated with the active transposing state. These alterations in the ability to transpose were transmitted through the germ line. McClintock[76] suggested that environmental stress may initiate transposition and thereby increase genetic variation and reshape the genome. If restructuring of the genome in response to stress does occur, it may be initiated by heritable epigenetic modifications (e.g. changes in DNA methylation) of transposable and other genetic elements which enhance the normal rate of transposition and result in an increased frequencyof base sequence changes.[77]

Transposition is not the only genomic change in plants which appears to be increased by stress.[78] Heritable changes in the DNA content and numberof copies of certain genes have been found in some flax varieties after growing the plants in a different environment for a single generation.[79] Cullis[80] has suggested that chromatin structure rather than DNA sequence is important in determining which genes are affected.

(3) Meiotic Recombination

It has been known for many years that the frequency of recombination in heterochromatic regions of chromosomes is lower than that in euchromatin.[81] Heterochromatin and euchromatin refer to conformational states which can be reversed[46] and are associated with differences in the degree of condensation of the chromatin, differences in functional states, and differences in the time of DNA replication.[82] If changes in chromatin conformation are induced in the germ line, the frequency of recombination in the affected region may be changed.

If the preceding arguments about the effects of chromatin structure on mutation and recombination are correct,

they suggest an additional way in which heritable epigenetic variations can have evolutionary significance. If the extent of sequence variation is partially determined by the gene's phenotype, it means that heritable epigenetic variations can influence the opportunities for Darwinian selection of mutations within the same chromosome region.

Conclusions

Our hypothesis suggests that inherited epigenetic changes in the structure of chromatin may play an important role in evolution. Accordingto the hypothesis, acquired epigenetic characters can be inherited and are involved in adaptive evolution and in speciation. Jacob expressed a generally accepted view when he said:

> "For modern biology, there is no molecular mechanism enabling instructions from the environment to be imprinted into DNA directly, that is, without the roundabout route of natural selection. Not that such a mechanism is theoretically impossible. Simply it does not exist"[83].

The arguments we have presented suggest that this view may not be entirely correct.

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