

Evidence, Mechanisms and Models for the Inheritance of Acquired Characters

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Several different types of epigenetic inheritance system enable alternative functional states to be maintained in cell lineages that have identical DNA sequences. Both random and guided (directed) epigenetic variations can be transmitted by these systems, and lead to heritable modifications in cell structure and function. Although it is usually assumed that epigenetic inheritance does not occur between generations, both old and new experimental evidence suggest, and in some cases show explicitly, that epigenetic variations can be transmitted from parents to progeny. Simple models of epigenetic inheritance in asexual and sexual organisms are presented. These show that in populations of asexual unicellular organisms, the distinctive properties of induced epigenetic variations mean that the variations may be retained for many generations after the inducing stimulus is removed, even in the absence of selection. The models also show that the epigenetic systems enable some types of acquired character to be inherited in sexual, as well as asexual, organisms. The importance of epigenetic inheritance systems in the evolution of multicellularity is discussed.

1. Introduction

Epigenetic inheritance systems (abbreviated EIS by Maynard Smith, 1990) are responsible for the stable inheritance of functional states in cell lineages. They are the reason why when fibroblast cells divide they give rise to fibroblasts, and when kidney cells divide they give rise to kidney cells. Differences between cells with identical DNA can persist and remain stable through many cell divisions, even when the stimuli that induced these differences are no longer present.

We have suggested previously that epigenetic inheritance systems are important not only in ontogeny, but also in phylogeny (Jablonka & Lamb, 1989). They can lead to “Lamarckian” inheritance. Our arguments were based on the biological properties of some EISs, on evidence showing that chromatin structure, and not just DNA sequence, is transmitted between generations, and on data indicating that some epigenetic variations can be passed from parents to offspring. In this paper we review the evidence suggesting that epigenetic information can be transmitted through many generations, and present some models of epigenetic inheritance systems. On the basis of the evidence and models, we discuss the evolutionary significance of inherited epigenetic changes, and the role of EISs in the evolution of multicellularity.

2. Epigenetic Inheritance Systems

EISs are inheritance systems which are additional to the familiar inheritance system based on DNA replication. Whereas we understand quite a lot about the enzymatic machinery and processes underlying DNA replication, we know less about the molecular mechanisms underlying epigenetic inheritance. What we do know is that there are several different types of epigenetic inheritance systems. We shall briefly describe three:

- (i) Chromatin-marking systems
- (ii) Steady-state systems
- (iii) Structural inheritance systems.

(i) *Chromatin-marking systems*

Chromatin is a complex of DNA, RNA and proteins. The functional state of a gene is related to the components of its chromatin and their conformation. A given chromatin region can have several alternative structures, which reflect different functional states: stably active, stably inactive, transiently active, inactive but easily inducible, etc. We have called the different alternative chromatin conformations that a gene can assume the *gene's phenotypes* (Jablonka & Lamb, 1989). Chromatin characteristics which are associated with the alternative gene phenotypes include the degree of condensation of the chromatin, the timing of DNA replication in S phase, the sensitivity of the chromatin to endonucleases, the amount of DNA modification by methylation, etc. Many studies have shown that these chromatin properties can be stably inherited in cell lineages (Conklin & Groudine, 1984; Weintraub, 1985; Van Holde, 1988; Holliday, 1990).

The best understood chromatin-marking EIS involves the inheritance of DNA modification patterns. Methylation, in which one of the bases of DNA has a methyl group added to it, is the most common modification. In eukaryotes, the methylated base is usually cytosine. The addition of a methyl group does not change the coding properties of the codon in which the base participates, but the extent of DNA methylation is related to the functional state of a gene (for reviews see Cedar, 1988; Holliday, 1990). Usually a low level of methylation is associated with potential transcriptional activity, a high level with inactivity. The same DNA sequence can have several different methylation patterns, each pattern being related to a different functional state.

Methylation patterns can be stably inherited through many cell divisions. Since methylation of cytosine in eukaryotes usually occurs in CpG doublets (or CpNpG triplets, where "N" is any base), methylation sites and patterns are symmetrical on the two strands of the DNA duplex. Consequently, after replication of a methylated site, each parental strand is methylated, but the new strand is unmethylated. Methyltransferase recognizes this asymmetrical state and methylates the CpG on the new strand. Thus the former state of methylation is reconstituted, and the methylation pattern is perpetuated. The fidelity of transmission from one cell to its daughter cells is over 99% for some sites, but it varies from site to site and locus to locus (compare Harland, 1982; Wilson & Jones, 1983; Yen *et al.*, 1986).

Models similar to that proposed for the inheritance of methylation patterns have been suggested to explain the propagation of other chromatin modifications such as those involving DNA-protein interactions. For example, Groudine & Weintraub (1982) proposed that protein subunits are symmetrically bound to the two DNA strands, and that after replication, the parental strand retains the bound protein subunits; the semi-bound state of the duplex is then a preferential site for the assembly of free subunits which bind and restore the original structure.

This type of EIS is one in which the chromosomes are the vehicles that carry epigenetic information from one generation to the next; the EIS operates by exploiting the semi-conservative replication of DNA. What is transmitted are chromatin "marks", and there is a special maintenance machinery ensuring that marks are replicated when DNA replicates. The system is autonomous in the sense that the maintenance of the functional state is independent of the nature of that state. The EIS enables permanent differences between homologous loci or homologous chromosomes, such as the differences between active and inactive X chromosomes in female mammals, to be established and maintained. Since the fidelity with which functional states are transmitted can be very high, it is not surprising that there are many cases where a hereditary variation that was at first assumed to be a variation in DNA sequence, was later shown to be an epigenetic variation (Holliday, 1987; Harris, 1989). The models we describe later are based mainly on this chromatin-marking type of EIS.

(ii) *Steady-state systems*

In "steady-state" inheritance systems, alternative cellular states persist as a result of the operation of self-regulatory feedback loops (Nanney, 1958). This type of system has two stable states, for example "on" and "off". Examples are shown in Figs 1 and 2. Figure 1(a) shows how a gene, once activated by an external inducer, can maintain its state of activity. Gene A produces product a which is lost or used at a rate k . The gene product positively regulates its own production by binding to a regulatory element of the gene coding for it. If the rate of production of a is greater than the rate at which it is lost or used, the system is in a stably active state. When the rate of loss is greater than the rate of production, the system switches to stably inactive. If p is the concentration of gene product a , $f(p)$ is the rate of production from gene A , k is the rate of loss of a (which is constant), then

$$dp/dt = -kp + f(p).$$

The system will have two stable states because there is a threshold concentration s such that

$$\text{if } p < s, \quad f(p) < kp$$

and

$$\text{if } p > s, \quad f(p) > kp.$$

Figure 1(b) gives an example of how such a system might operate over time. Many genes seem to autoregulate in this way, for example, the mammalian proto-oncogene

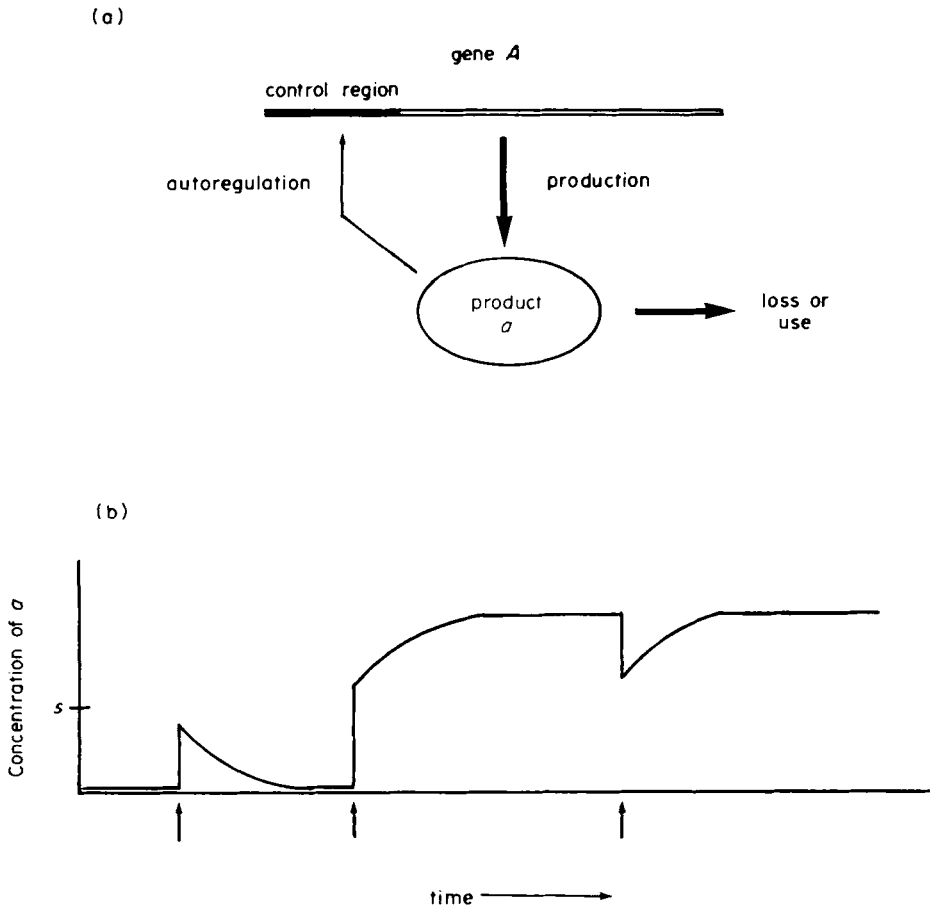


FIG. 1. (a) A steady-state system involving autoregulation of gene *A* by its own product *a* (see text for details). (b) The type of behaviour expected from a steady-state system such as that shown in (a). At the times indicated by arrows, a stimulus which changed the concentration of product *a* was introduced. *s* is the threshold concentration above which $f(p) > ks$.

c-onc and the *Drosophila* homeotic gene *fushi tarazu* (Serfling, 1989). Figure 2 shows that a gene does not have to regulate *itself* for a functional state to persist. If two or more genes are associated via a positive regulatory loop, the functional state will be maintained. The primary stimulus activates gene *A* to produce the product *a*, which activates gene *B*, whose product *b* activates gene *A*. From the moment this state is established, both genes *A* and *B* can remain active, even in the absence of the stimulus which originally induced gene *A*. The equations describing this system are comparable to those of the simpler system described earlier:

$$dp_a/dt = -k_a p_a + f_a(p_b)$$

$$dp_b/dt = -k_b p_b + f_b(p_a).$$

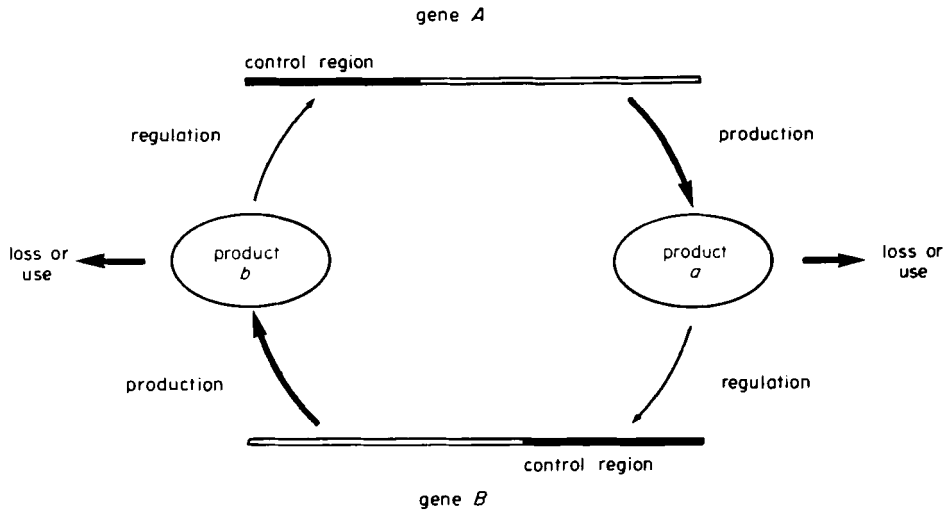


FIG. 2. A two gene steady-state system. The product *a* of gene *A* activates gene *B*, whose product *b* activates gene *A*.

Such regulatory networks probably underlie the reaction of ciliates to immobilizing antigens (Nanney, 1980), and seem to be characteristic of several genetic systems in eukaryotes (reviewed by Serfling, 1989). The networks allow transient stimuli, such as various morphogens, to have far-reaching and permanent developmental effects.

The property which is transmitted to daughter cells in these EISs is the concentration of the regulatory proteins. Assuming that there is a reasonable number of molecules of the proteins and they are distributed evenly in the cell, the "heritability" of functional states may be quite high. There is no special maintenance machinery, such as is required for a chromatin-marking EIS; the inheritance of a functional state is a simple consequence of the operation of the homeostatic system and of a more or less equal cell division. This type of EIS depends critically on the self-regulatory functions of the gene products involved. The systems can easily be perturbed by any factors which change the intracellular concentrations of the regulators.

(iii) *Structural Inheritance systems*

In *Paramecium* and other ciliates, Sonneborn and others have shown that pre-existing cortical structures serve as templates for new structures in daughter cells (Beisson & Sonneborn, 1965; Ng, 1990). The cortex of these unicellular organisms is very complex and highly organized. If a cortical structure is damaged accidentally, or changed experimentally by microsurgery, the cell may transmit the modified structure to daughter cells. What is inherited therefore depends not only on information coded in DNA and embedded in the gene's phenotype, but also on the architecture of previous structures. The EIS allows variations in architecture, which involve neither changes in DNA sequence nor in gene function, to be inherited for many generations.

This type of inheritance is common in ciliates, but it is not clear how important it is in other organisms. There are indications that something similar exists in the flatworm *Stenostomum incaudatum* (Sonneborn, 1930) and in the protozoan *Diffugia corona* (Jennings, 1937). It may also be a more general feature of cellular heredity, since in addition to the templating activity associated with the multiplication of centrioles, features showing architectural continuity have been found in neuroblastoma and 3T3 cells *in vitro* (Albrecht-Buehler, 1977; Solomon, 1979), and caterpillar epidermis cells *in vivo* (Locke, 1988).

We have described the chromatin-marking, steady-state and structural EISs as if they are independent of each other. Although often they may be, they can also be interconnected and interdependent. For example, the inheritance of chromatin conformation may involve architectural continuity if the old chromatin strand acts as the template for the new strand as Groudine & Weintraub (1982) suggest. Similarly, the chromatin-marking and steady-state systems are interconnected because the inheritance of the methylation pattern at a methylase locus must depend on the activity of that locus.

3. Epigenetic Variations

The variations which are transmitted by epigenetic inheritance systems can be either "random" or "guided". We are using "guided" where others have used "directed" because we want it to be clear that the variations are guided by the internal or external environment, but are not necessarily *directed towards an end*. A variation is "guided" when it is specifically induced by the environment. During ontogeny, such specific, induced epigenetic variations are crucial for determination and differentiation. A stimulus affects a specific gene (or cell structure) in a particular cell type at a particular stage of development. It has no consistent effect on other genes, or on the same gene in a different tissue or at a different developmental stage. The frequency of such guided variations is 100% in some cell types at some developmental stages. Likewise, the frequency of "reversion" can be very high.

Epigenetic variations can also be "random". Random variations are non-specific with regard both to the stimulus that induced them, and the gene which is modified. Holliday (1987) termed such hereditary epigenetic modifications "epimutations". The frequency with which epimutations are induced is higher than the frequency of induction of classical mutations (Jablonka & Lamb, 1989; Holliday, 1990).

The difference between the frequency with which epimutations and mutations occur is not the only important difference between the two inheritance systems. From an evolutionary point of view, it may be more important that, unlike genetic changes, epigenetic changes are likely to occur co-ordinately in several loci at the same time. Moreover, the same guided variations are likely to occur in a number of different individuals if they are exposed to the same environmental conditions.

4. The Transmission of Epigenetic Variations

It is clear that EISs are very important in development. Once the environment or the developmental system has induced a change in a group of cells, that change

is maintained and transmitted without any further need for the environmental or developmental stimulus. The EIS frees the system from its continuous dependence on specific stimuli. It ensures the stability of transmission of determined states and hence the stability of development.

The role of EISs in phylogeny is less clear. Most biologists deny that epigenetic inheritance has any direct importance in evolution. They assume that epigenetic variations cannot be transmitted between generations. However, there is evidence suggesting that this widely held opinion is incorrect. Some of this evidence is summarized in Table 1, which shows that epigenetic inheritance between generations can occur in both asexual and sexual organisms.

5. Epigenetic Inheritance in Asexual Organisms

In unicellular asexual organisms, cellular heredity is identical with between-generation heredity, and it is often difficult to distinguish between genetic and epigenetic inheritance. In some cultured cell lines, hereditary variations which were initially assumed to be classical mutations, turned out to be epimutations (Holliday, 1987; Harris, 1989). The old literature of genetics contains descriptions of unorthodox patterns of inheritance in unicellular organisms which may also turn out to involve epimutations. The hereditary variations described are neither transient, like some somatic modifications of gene expression, nor as stable as classical changes in DNA sequences. Jollos (1921) called this class of variations "Dauermodifikationen", or "lingering modifications". He found that in lines of *Paramecium* initiated from a single cell, changes induced by a specific environment (e.g. by temperature, by high salt concentrations, by exposure to arsenic), are inherited and persist through asexual reproduction long after the removal of the inducing stimulus. Gradually the modifications fade away until finally, after hundreds of generations, they disappear. Typically, such modifications disappear immediately after sexual reproduction, but this is not always the case. For example the effects of calcium were not reversed after a single round of sexual reproduction; it took a substantial amount of time or several consecutive conjugations to reverse the effect.

The results of Jollos's study of lingering resistance to arsenic are summarized in Table 2. Similar results were obtained for resistance to high concentrations of salt. The experiments have been repeated and the results confirmed by later workers (cited by Beale, 1954), but the mechanisms underlying lingering modifications remain unclear. We believe that epigenetic inheritance provides a plausible explanation of Dauermodifikationen. The following models describing the inheritance of epigenetic marks between generations in asexual organisms seem to fit the type of data obtained by Jollos.

5.1. MODELS OF EPIGENETIC INHERITANCE IN ASEQUAL ORGANISMS

In the models we assume:

- (1) A given gene can have a certain finite number of mark-configurations, or phenotypes.

TABLE 1

Examples of transgenerational inheritance that may have an epigenetic basis

Organisms	Type of heritable variation	Type of EIS	References
<i>Paramecium</i> (protozoan)	Induced responses to temperature, salt, and arsenic (Dauermodifikationen)	N.K.	Jollos (1921)
<i>Paramecium</i> , <i>Tetrahymena</i> , <i>Stylonichia</i> , <i>Paraurostyla</i> , <i>Euplotes</i> (protozoa)	Accidental or induced variations in cortex structure	Structural	Sonneborn (1964), Nanney (1968), Nelsen <i>et al.</i> (1989), Ng (1990)
<i>Diffugia corona</i> (protozoan)	"Teeth" structure‡	Structural	Jennings (1937)
<i>Saccharomyces cerevisiae</i> (yeast)	Utilization of melibiose‡	N.K., but probably steady state	Spiegelman <i>et al.</i> (1945)
	Transcriptional state of HML α gene	N.K., but chromatin marks suggested	Pillus & Rine (1989)
<i>Aspergillus nidulans</i> (fungus)	Fluffy phenotype induced by 5-aza-cytidine	Chromatin marks methylation pattern	Tamame <i>et al.</i> (1988), Tamame & Santos (1989)
<i>Coprinus cinereus</i> (fungus)	Methylation pattern at the 16.1 locus	Chromatin marks methylation pattern	Zolan & Pukkila (1986)
<i>Pisum sativum</i> (pea)	Induced response to temperature	N.K.	Highkin (1958a, b)
<i>Phaseolus vulgaris</i> (bean)	Induced response to temperature	N.K.	Moss & Mullett (1982)
<i>Oryza sativa</i> (rice)	Induced dwarfism and reduced level of methylation	Chromatin marks methylation pattern	Sano <i>et al.</i> (1989, 1990)
<i>Zea mays</i> (maize)	Transposition of <i>Spm</i> , <i>Ac</i> , <i>Mu</i>	Chromatin marks methylation pattern	Fedoroff <i>et al.</i> (1989a, b), Denis & Brettell (1990), Martienssen <i>et al.</i> (1990)
	Paramutation in the R locus†	N.K.	Brink (1973)
<i>Nicotiana tabacum</i> (tobacco)	Requirement of leaf cells for cytokinin	N.K., but steady state suggested	Meins (1985, 1989)
	Expression of genes on T-DNA	Chromatin marks methylation pattern	Matzke & Matzke (1990)
<i>Lolium perenne</i> (perennial ryegrass)	Colchicine-induced variation	N.K.	Francis & Jones (1989)
<i>Triticum</i> (wheat)	Cytosine methylation of glutenin genes	Chromatin marks methylation patterns	Flavell & O'Dell (1990)
Many species of plants	Developmental phase‡	N.K.	Brink (1962)
<i>Stenostomum incaudatum</i> (flatworm)	Induced resistance to lead acetate‡	N.K.	Sonneborn (1930)
<i>Pieris brassicae</i> (butterfly)	LSD-induced resistance to LSD and changes in diapause	N.K.	Vuillaume & Berkaloff (1974)

TABLE 1—continued

Organisms	Type of heritable variation	Type of EIS	References
<i>Philodina citrina</i> , <i>Euchlanis triquetra</i> (rotifers); <i>Drosophila melanogaster</i> (fruit fly); <i>Poecilia reticulata</i> (fish)	Lansing effects—various characters show cumulative progressive changes with parental age†	N.K.	Lansing (1954), Lints (1978), Beardmore & Shami (1985), Jablonka & Lamb (1990)
<i>Mus musculus</i> (mouse)	Expression of <i>fused</i> gene†	N.K.	Belyaev <i>et al.</i> (1981, 1983), Ruvinsky (1988)
	Expression and methylation of hepatitis B surface-antigen transgene	Chromatin marks methylation pattern	Hadchouel <i>et al.</i> (1987)
	Expression and methylation of TKZ751 transgene	Chromatin marks methylation pattern	Allen <i>et al.</i> (1990)
Rats, mice, guinea-pigs, rabbits	Haemoglobin level	N.K.	Kahn (1982)
	Drug and hormone, induced changes in endocrine function	N.K.	Campbell & Perkins (1988)
<i>Homo sapiens</i> (man)	Methylation of endogenous sequences	Chromatin marks methylation pattern	Silva & White (1988)

† Similar observations have been made in other organisms: see Lints (1978) for a review of Lansing effects, Brink (1973) for paramutation, Ruvinsky (1988) for an account of characters in foxes which behave similarly to *fused* in the mouse.

‡ The variation is propagated only in asexual reproduction.

N.K. = Not known.

(2) In the absence of an inducing stimulus (the normal state), there are certain probabilities that a mark will be transmitted to the next generation unchanged, or changed to another mark [Fig. 3(a)].

(3) In the presence of a stimulus, the probabilities change, so that some gene phenotypes are "induced" [Fig. 3(b)].

To simplify the model, a fourth assumption has been made. It is a mathematical, rather than a biological, assumption.

(4) In the absence of a stimulus, the transition from one gene phenotype to another is progressive, e.g. the gene cannot change from an unmethylated to fully methylated state or vice versa in a single step.

5.1.1. The two-state model

The simplest case of inherited epigenetic marks occurs when a gene can have only two phenotypes. In this case, the equation describing the changes in mark frequency in a population is exactly the same as that describing changes in allele frequency as a result of mutation pressure. If we assume that a given sequence can have two

TABLE 2

The inheritance of resistance to arsenic in Paramecium previously exposed to arsenic (based on Jollos, 1921)

Experiment, strain and conditions	Resistance of untreated strain (%)†	Resistance following treatment‡	Days before reversion to the original sensitivity§
Expt 1 B	1	5	259
Expt 2 α	0.9	3.5	164
Expt 3 Z	0.8	3.5	145
Expt 4 B	1	6	317
Expt 5 A	0.9	4	125
with environmental fluctuations			48
Expt 6 α	0.9	3.5	136
with conjunction on day 18			Immediate

† Highest percentage of a 0.1 N solution of As_2O_3 in which they could survive.

‡ Paramecia, which had two to three generations per day, were treated with increasing concentrations of arsenic for short periods, with intervals of 2–3 days between treatments. For example, in Expt 1 the treatment periods were 10 hr in 1.5%, 12 hr in 1.5%, 10 hr in 2%, 12 hr in 2.5%, 2 hr in 3%, 12 hr in 2% and 24 hr in 2.5%. When resistance to higher arsenic concentrations was discerned, the culture was returned to an arsenic-free environment, and periodically checked for resistance.

§ In general the reversion was gradual, although sometimes a culture suddenly decreased in resistance.

|| After resistance had developed, the culture was grown in arsenic-free medium, but in fluctuating temperature and nutritional conditions.

marks, m_1 (the original mark) and m_2 , which have characteristic rates of change from one to the other, then the change in the frequency of marks will be:

$$p_t = \frac{v}{u+v} \left(p_0 - \frac{v}{u+v} \right) (1-u-v)^t$$

where u is the rate of change from m_1 to m_2 , v is the rate from m_2 to m_1 , and p_0 and p_t are the frequencies of m_1 at generation 0 and t .

The important difference between changing the frequency of marks and changing the frequency of alleles is that whereas mutation pressure changing allele frequency is weak because u and v are very low, the pressure of epigenetic change can be considerable. Figure 4 illustrates how the frequency of marks changes following a brief exposure to an environmental stimulus which increases the rate of induction of mark m_2 from $u = 5 \times 10^{-3}$ to $u = 0.6$, while leaving the reversion rate unchanged ($v = 10^{-2}$). It shows that after the removal of the stimulus and return to the "normal environment" ($u = 5 \times 10^{-3}$, $v = 10^{-2}$), m_2 "lingers" on, and the population only gradually reverts to its previous condition. Even when $u = 0$, the mark may persist for a while; if the altered mark has a selective advantage, a balance between selection and reversion may be reached.

5.1.2. The multi-state model

The multi-state model describes a progressive change in the induced state. A cell can be in n epigenetic states, and we shall describe the case when $n = 4$, $m_1 - m_4$ may

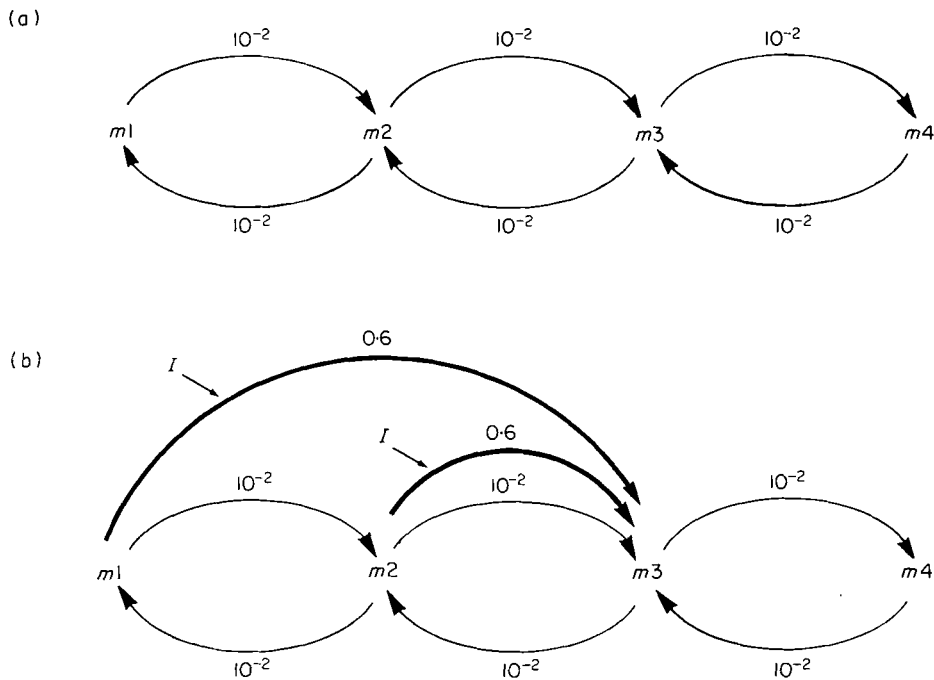


FIG. 3. Examples of changes in marks $m_1 - m_4$ in the presence and absence of an inducer. (a) In the absence of an inducer each mark can change into the subsequent or previous mark, with a probability of 10^{-2} . (b) In the presence of the inducer I , m_1 and m_2 are changed to m_3 with a probability of 0.6, but the spontaneous rates of mark change remain as in (a).

represent four phenotypes of a gene (four types of marks), or they may represent four functional states of a cell with several genes involved. In order to simplify the discussion, we shall talk of the state of a single gene. States m_1 and m_2 are "inactive" states of the gene, differing in their marks (e.g. methylation states): m_1 is more stably inactive than m_2 ; states m_3 and m_4 are active states, which again differ in their marks. Figure 3 is an example of such a system with the fidelities of transmission from one state to the other specified. Figure 5 shows the change in the frequency of active genes with time, starting with a population in which none of the genes is active. It can be seen that in the absence of an inducing stimulus [Fig. 5(a)], the population slowly approaches equilibrium, with half the genes active and half inactive. Figure 5(b), (c) and (d) shows what happens when an inducer is present for one, two, and five generations, again starting from a situation in which all genes are inactive. It can be seen that after even a single generation of exposure to an inducing stimulus, a high proportion of the genes become active, and this state persists for many generations. A longer exposure to the inducing conditions results in more active genes, and the induced state lingers for even longer. It should be noted that in none of the examples illustrated in Fig. 5 is any selection for or against the induced state acting on the populations.

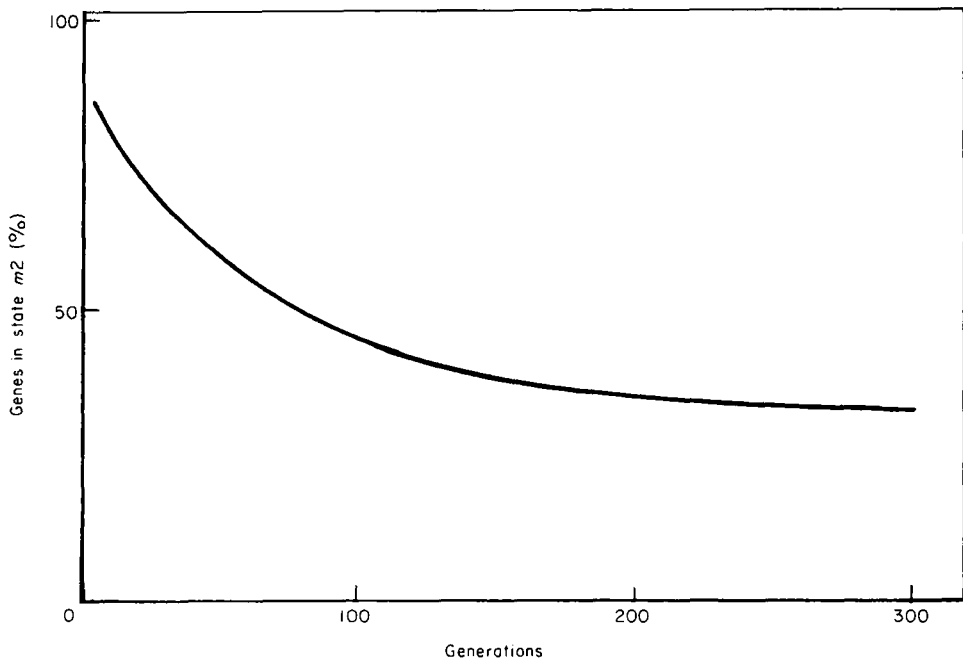


FIG. 4. Change in mark frequency for a gene which can exist in two states, m_1 and m_2 , following exposure for two generations to an inducer of m_2 . (For details, see text.)

The models can also be applied to developmental (intra-generation) processes in multicellular organisms where, once induced, the determined and differentiated states are maintained, even in the absence of the inducing stimulus. With modifications, the models can be applied to sexually reproducing organisms, but only if it is assumed that gametogenesis and meiosis do not alter the simple rules of cellular heredity.

6. Epigenetic Inheritance in Sexual Organisms

It can be argued that although heritable epigenetic variations can occur, they are of limited importance because they cannot be stably inherited in sexually reproducing organisms. It is generally believed that all, or most, epigenetic information is erased when the germ-line cells differentiate into gametes, so that the fertilized egg always starts from "square one". However, evidence for the transfer of one type of epigenetic information through the germ-line has been known for many years. The evidence comes from the transmission of sex-specific information seen in genomic imprinting, where the expression of some loci, whole chromosomes or genomes, depends on their parental origin (Jablonka & Lamb, 1989). For example, when an allele comes from mother it is inactive, whereas when it comes from father it is active. The DNA sequence of the allele remains the same, but when passing through gametogenesis, the DNA acquires sex-specific marks which are transmitted to the progeny.

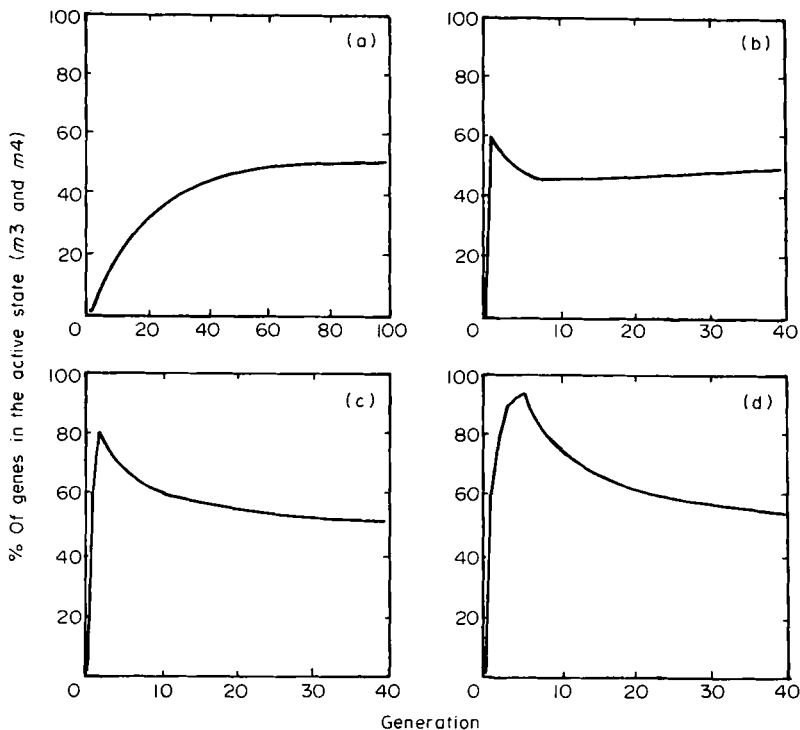


FIG. 5. Frequency of genes in the active states ($m3$ or $m4$) for the four-state model described in the text and Fig. 3(b). Initially, at generation 0, all genes are in state $m1$. An environmental stimulus causes genes in the inactive states ($m1$ and $m2$) to change to state $m3$ with a probability of 0.6 [see Fig. 3(b)]. In (a) there is no stimulus; (b) the stimulus is present for one generation; (c) the stimulus is present for two generations; (d) the stimulus is present for five generations. Note the different time scale in (a).

In genomic imprinting, although the mark of the parent is transmitted to the offspring, the mark carries only the stamp of the parent's sex. No other aspect of the parent's phenotype is retained. Moreover, the mark is transient and is reversed in the next generation if it passes through the germ-line of the opposite sex. Imprinting is therefore a rather special case of the transmission of epigenetic marks between generations. Cases where an epigenetic mark is transmitted in a sex-independent fashion and in a more permanent manner are needed to illustrate the operation of EISs between generations. Possible examples of such cases are summarized in Table 1. These can all be interpreted as instances of epigenetic inheritance, although only in the more recent studies is there direct molecular evidence for epigenetic inheritance. We shall briefly review some of the cases for which molecular evidence is available.

Flavell & O'Dell (1990) showed that in an inbred line of wheat, where all individuals had identical DNA sequences for the gene coding for the high molecular weight glutenin protein, plants differed in the methylation patterns imposed on the sequence. Five methylation variants were found, and their patterns of methylation were stably inherited both somatically and through meiosis. In crosses between methylation

variants, the F_1 generation had patterns from both parents, and in the F_2 the variants segregated so that the parental and F_1 types were recovered. In addition, new patterns were sometimes observed. These data show that methylation patterns are inherited and marks segregate with the DNA sequence on which they are imposed. The appearance of new variants demonstrates the relatively high "epimutability" of this chromatin region. A similar phenomenon has been found in the fungus *Coprinus cinereus* where it was shown that methylation patterns at a centromere-linked locus are inherited (Zolan & Pukkila, 1986). The most decisive demonstration that these methylation variants behave like Mendelian markers was obtained by crossing two strains identical in their DNA sequence, but differing in their methylation pattern. Tetrad analysis revealed a 2:2 segregation, exactly as is obtained for classical Mendelian alleles differing in DNA sequence.

Experiments which link the inheritance of environmentally induced characters with the inheritance of methylation patterns have been reported by Sano *et al.* (1989, 1990). They showed that a single exposure of germinated rice seeds to the demethylating agent 5-azacytidine (or to 5-azadeoxycytidine) induces a high frequency of dwarfism. At the molecular level, they found substantial demethylation of total DNA. The dwarf phenotype was inherited for at least three generations and the low level of methylation induced by the treatment segregated with the dwarf phenotype. Sano *et al.* postulated a direct causal relationship between the inheritance of stature and the inheritance of the level of methylation: they suggested that 5-azacytidine treatment demethylates loci relevant to stature, and these undermethylated loci and the associated dwarf phenotype are transmitted to the next generation. A similar phenomenon has been described for *Aspergillus* where treatment of cells with 5-azacytidine caused a highly specific induction of the *fluffy* growth phenotype. This phenotype was mitotically and meiotically stable. In spite of the generally low level of methylated bases in the *Aspergillus* genome, there are some methylated sites, and the suggestion that 5-azacytidine-induced demethylation caused the *fluffy* phenotype is plausible (Tamame *et al.*, 1988; Tamame & Santos, 1989).

Evidence that epigenetic information can be transmitted through the germ-line has also come from Silva & White's (1988) studies of human tissues. They showed that, in some tissues, the methylation patterns at two allelic sites differ. The variants are inherited in a Mendelian fashion for at least three generations. The allele-specific methylation patterns are not, however, preserved in the sperm, where the pattern at the locus is uniform and sperm-specific. Therefore, in this case the variations in methylation pattern are not transmitted directly, but some blueprint of them must be established during gametogenesis. Silva & White suggested that elements such as DNA binding proteins, which segregate with the chromosomes at meiosis, may serve as the blueprints.

The regulation of transposition in maize is one of the most thoroughly investigated cases showing a relationship between heritable methylation patterns and phenotypic variations (reviewed in Fedoroff *et al.*, 1989a, b). Some heritable variations in transposable elements have been shown to be epigenetic variations, not changes in DNA base sequence. The differences between elements with different "transposabilities" are strongly correlated with their heritable methylation patterns. For the *Spm*

(*Suppressor-mutator*) element, the heritable changes which inactivate it are reversible, quantitative and progressive. This element can exist in three interconvertible states: (i) *cryptic*—a stably inactive state, associated with substantial methylation of both downstream (DCR) and upstream (UCR) control regions; (ii) *programmable*—a state that is altered during development; an inactive programmable element is inactive, but easily reactivated, and this is associated with methylated UCR and partially unmethylated DCR; an active programmable element is active, but prone to inactivation, having an unmethylated UCR and less methylated DCR; (iii) *active*—a stably active state, associated with the lowest levels of methylation in UCR and DCR. The likelihood of a change in the state of activity of a programmable element depends on the presence or absence of other active elements in the genome, the sex of the gamete that transmits the element, and the part of the plant that produces the gametes. Fedoroff *et al.* (1989b) concluded their analysis of *Spm* regulation and transmission: “perhaps the most striking observation that has emerged from the analysis of the *Spm* element’s developmental control mechanism is that epigenetic changes in the present generation can influence the expression pattern of the element in the next generation”.

DNA methylation is also a component in the control of other transposable elements in maize, such as the *Ac* element (Chomet *et al.*, 1987; Kunze *et al.*, 1988) and the *Mu* element (Chandler & Walbot, 1986; Martienssen *et al.*, 1990). Like the *Spm* element, their activity state, which is associated with DNA methylation, can be altered during development, and the altered state can be transmitted to the next generation (Dennis & Brettell, 1990).

Inherited gene activity which is associated with heritable methylation levels is also seen in transgenic tobacco plants, where marker genes from one T-plasmid (T-DNA-I) can become inactivated when cells are transformed by a second T-plasmid (T-DNA-II) (Matzke *et al.*, 1989). T-DNA-II induces methylation as well as inactivation. When plants are selfed and the two T-DNAs segregate so that T-DNA-I is not in the company of T-DNA-II, T-DNA-I is reactivated and demethylated. However, the demethylation is not complete in the first generation, where individual plants have two populations of cells, some with active and demethylated T-DNA-I, and others in which it is methylated and inactive. As with *Spm* regulation, the activity of the T-DNA-I gene depends on the presence or absence of another element (T-DNA-II) and on its own methylation status. These experiments led Matzke & Matzke (1990) to suggest that the phenotypic variability observed in plants with identical genotypes may be due to epigenetic, potentially heritable variations, and that somatic selection of epigenetic variants in the meristem enables plants to adapt rapidly to changed environmental conditions, without waiting for genotypic change.

In transgenic mice, as in transgenic tobacco plants, the expression of a locus depends both on its level of methylation and on the effects of modifiers. Allen *et al.* (1990) showed that the expression and methylation level of a transgene locus (TKZ751) is affected by its chromosomal position and by the genetic background. On a BALB/c background the locus was inactive and heavily methylated (providing the BALB/c genes were maternally transmitted), whereas on a DBA/2 background its expression was enhanced, and methylation was reduced. The methylation level of

the locus changed progressively over successive generations of selection: it became completely methylated after three generations of selection on a BALB/c background, and almost completely demethylated after three generations of selection on DBA/2 background. Passage through the germ-line obviously did not erase the epigenetic information acquired previously, for had it done so, such cumulative effects would not be seen. Once the locus became fully methylated, it retained this state and remained inactive even when put on the DBA/2 background, i.e. the fully methylated state had become irreversible and self-propagating. A similar result was obtained with another transgene in the mouse which became irreversibly inactive after passage through female gametogenesis (Hadchouel *et al.*, 1987).

Clearly, there is a substantial amount of direct evidence for the inheritance of both random and environmentally induced epigenetic variations. In spite of the radical changes in chromatin structure which take place during meiosis and gametogenesis, not all of the epigenetic information acquired during the lifetime of an organism is erased before or during gamete formation. For some genes, marks reflecting their past activity remain in a form which can be interpreted in the next generation. Even when its DNA sequence remains unchanged, a gene is not always reset to the same epigenetic state during gamete production.

6.1. MODELS OF EPIGENETIC INHERITANCE IN ORGANISMS WITH A SEGREGATED GERM-LINE

Maynard Smith (1990) has presented models showing how an EIS might work in organisms in which the epigenetic state is reset in the germ-line. In these models, genes switch between a somatic cell state and a germ cell state in response to external inducers. The inducers combine with regulatory proteins and activate the genes producing enzymes which change marks. The model is based on several assumptions including (i) that each mark can exist in two states, soma-specific and germ-line-specific, with developmental signals switching the system from one state to the other; (ii) that the normal germ-line state is a unique and invariant state. With these assumptions, an epimutation in the soma cannot be stably inherited, because in the next transition from soma to germ-line, the mark will be restored to the original germ-line state. Similarly, epigenetic errors in the germ-line will not be transmitted stably, because the germ-line will reset to its original condition in the next generation. Maynard Smith concluded from his models that the resetting which takes place in the germ-line means that epigenetic changes cannot be of direct importance in evolution unless they are accompanied by a DNA sequence change.

We believe that this conclusion is incorrect because there are flaws in the assumptions on which Maynard Smith's model is based. The assumptions that a mark can have only two states and that the variations in the germ-line state are not heritable are misleading oversimplifications. The evidence outlined in this section shows that a locus may exist in several epigenetic states, and that the germ-line is

not always reset to the same state. In Fig. 6 we describe what we believe is a more plausible model for the transmission of epigenetic marks, and show the consequences of altering them. Figure 6(a) depicts the "normal" situation in which the marks on two genes are modified during development, but are reset to the original state in the germ-line, so that the developmental cycle of marks is repeated in the next generation. The marks carried by genes *A* and *B* are different, and they respond to, and are changed by, different inducers. In Fig. 6(b) and (c) we show what may happen when a mark on gene *A* is altered either as a result of a random change, or in response to an environmental inducer. In Fig. 6(b), the situation is similar to that described by Maynard Smith, since the new epigenetic mark on *A* is sufficiently like the old one to be recognized and reset in the germ-line to the normal germ-line-specific mark. Such epigenetic variations are not inherited. Figure 6(c) shows one way in which an altered epigenetic mark can be stably inherited: the new mark on gene *A* is such that it mimics the normal mark on gene *B*; consequently, the gene will change marks in the same way as *B*, and will be reset in the germ-line in the same way as *B*. A heritable epigenetic change has occurred. It is possible to imagine other outcomes of changed marks in addition to those shown in Fig. 6. For example, there could be a "domino effect" in which a new mark is not recognized by any of the existing programmes for changing marks, but instead undergoes a series of almost random changes, until ultimately a stable state is reached, or the organism dies.

The model described in Fig. 6(c) shows that the transmission of an altered epigenetic mark need not involve a change in DNA sequence, although such a change could further stabilize the inheritance of the new phenotype. If DNA sequence changes do not occur, the frequency of the new mark in the population will depend on the rate of acquisition and reversion of the mark, and on its effects on fitness. If the change in the mark is environmentally induced, the stability of the environment will obviously be important.

7. Discussion

What general conclusions can be drawn from the data and models of epigenetic inheritance? The first, and most important, is that epigenetic information is not totally erased in the germ-line; chromosomal marks—in the cases investigated at the molecular level, DNA methylation patterns—can be transmitted to the next generation. This is easily understood in the case of asexual organisms, where organismal continuity and cellular inheritance are tightly linked. The models for asexual organisms show that induced marks can persist in a population long after the inducing stimulus is removed. The length of time they linger depends on the efficiency of induction, the rate of reversion, and the rate of spontaneous mark acquisition. In sexual organisms the situation is more complicated. We do not yet know exactly what happens during gametogenesis and meiosis—how marks are altered, which marks are altered, whether marks which are altered during gametogenesis and meiosis usually, or rarely, leave "footprints" of their previous nature, and so on. We obviously need to know what processes occur in the germ-line in order to be able to assess the fidelity of inherited epigenetic variations and the type of locus affected. It

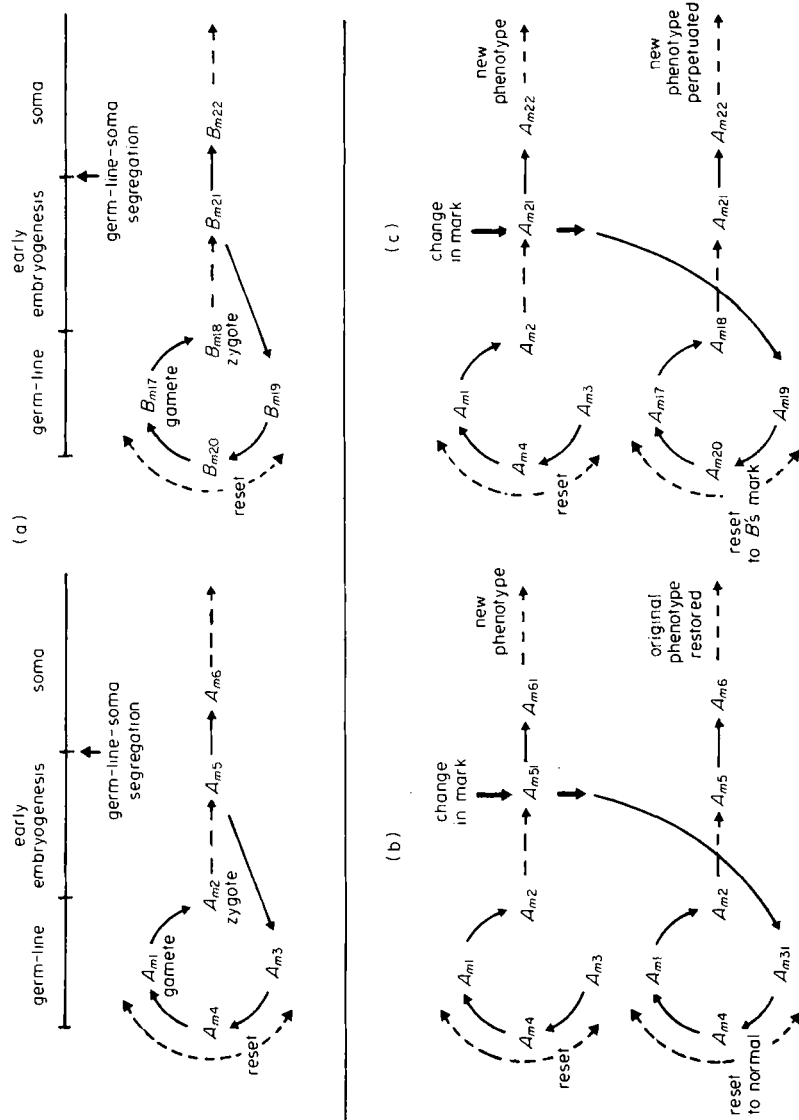


FIG. 6. Models of epigenetic inheritance. Each gene carries a mark (m) which changes during development. Different numbers represent different marks. (a) Changes in the marks on genes A and B during normal development, showing how developmental cycles are perpetuated. (b) An altered mark on gene A which does not lead to an inherited change. The normal mark $m5$ is altered to $m51$ and produces a new phenotype. The transition of $m51$ to the germ-line state results in a new mark $m31$. However, $m31$ is sufficiently similar to $m3$ for it to be reset to the normal mark $m4$. The normal cycle is thus restored and there is no memory of the epigenetic change. (c) An altered mark on gene B which results in an inherited variation. The normal mark $m5$ is changed to $m21$, a mark which mimics that normally found on B , and produces a phenotypic change. In the germ-line, like $m21$ on B , $m21$ on A is reset to $m20$. From this point onwards, marks on A are changed and inherited like those on B , and the new phenotype is perpetuated. In both (b) and (c), the change in mark is shown as taking place in early embryogenesis; it could equally well occur at any stage prior to germ-line-soma segregation and have the same effects. Since the EIS which we have in mind is the chromatin-marking EIS, we expect that when transmitted between generations, the chromatin marks will segregate in a Mendelian fashion, just like chromosomal variations in DNA base sequence. For ease of presentation, the contribution of the male parent has been omitted.

is clear though, that not only random, but also guided epigenetic variations can be inherited. It is also clear that the stability of the inheritance of epigenetic marks can vary: some variations persist for only a few generations, whereas others seem very stable.

In the cases which have been investigated at the molecular level, the heritable epigenetic mark studied has been the pattern or extent of DNA methylation. It is not clear whether DNA methylation is really the major mechanism of chromatin "marking", or whether heritable variations in DNA methylation have been detected simply because at present this is the only type of epigenetic mark we can study easily. There are no suitable techniques for the detailed study of DNA-binding-protein marks.

Although the data in Table 1 are not extensive enough to allow generalizations, it is interesting that many of the cases of epigenetic inheritance in multicellular organisms occur in plants. This may not be a coincidence. Only epigenetic marks which are present in the germ-line can be transmitted to the next generation. In organisms with late or non-existent soma-germ-line segregation, epigenetic variations that occur in somatic cells can be transmitted to the next generation because these somatic cells can become germ-line cells. As Buss (1987) and Klekowski (1988) have suggested for mutational variations, if a new epigenetic variation in a cell is advantageous at the tissue level, somatic selection can occur, and the cells with the new variation may come to dominate the tissue, so that their chance of being transmitted as germ cells increases. Thus, in organisms like plants, which do not have a segregated germ-line, new epigenetic variations in somatic lineages may be inherited. In organisms such as mammals, which have early soma-germ-line segregation, only epigenetic variations which occur during the early stages of ontogeny, before the segregation of germ-line and soma, or which occur in the germ lineage itself, can be transmitted to the next generation. This leads to the general prediction that the majority of cases of inherited epigenetic variations in somatic characters will be found in organisms with late or no sequestration of the germ-line.

Some of the more detailed studies of the inheritance of epigenetic variations (in maize, tobacco and mice) have shown that there are interactions between the marked sequence and other loci. This is not surprising. Epigenetic marks are expected to behave like mutations in *cis*-acting regulatory elements which alter the extent or specificity of gene expression. The genetic background is important because gene expression depends on the interaction between *cis*-elements and regulatory proteins. Some epigenetic variations will alter the binding affinity of *trans*-acting regulatory proteins, others will not. The *trans*-acting gene products of some alleles will be able to bind normally to a differently marked sequence, whereas the products of other alleles of the same gene may have radically changed affinities for the altered mark, and therefore have pronounced effects on gene expression. The interactions between marks and regulatory proteins mean that epigenetic changes affect the level of transcription. It is the *amount* of a gene's product that is affected, not the nature of that product. Consequently, the effects of new epigenetic variations will often be subtle "quantitative" changes, which are not as deleterious as the effects of classical random mutations.

The major objection to the proposal that the transmission of epigenetic variations is important in evolution is that, in spite of a lot of genetic work, very few cases of this type of inheritance have been found. There are many answers to this objection: (i) Most of the ideas about EISs and the experimental attempts to understand them are recent, and it is likely that many more examples will be found. (ii) Probably many inherited changes which are at present thought to be the result of conventional DNA sequence changes, will turn out to be heritable epigenetic variations. This occurred with inherited changes in cells in culture, where many "mutations" were found to be epimutations (Holliday, 1987; Harris, 1989). (iii) People have been looking for the wrong kind of manifestation of epigenetic inheritance. It is often implicitly assumed that the inheritance of epigenetic variations is synonymous with traditional "inheritance of acquired characters". It is not. Some epigenetic variations—epimutations—are random (in the biological sense), and will rarely produce adaptive characters. Guided epigenetic variations will lead to the inheritance of acquired characters only if they have phenotypic effects. Many guided changes in epigenetic marks will not change gene expression directly, but will affect the way that the expression of the gene is influenced by other factors. The more subtle effects on gene expression may be difficult to analyse because they appear as quantitative variations, which often behave erratically and show incomplete penetrance and variable expressivity. (iv) The phenomenon has been sought in the wrong place. People were looking for inherited somatic adaptations—the giraffe's neck, the colour of salamanders, the degeneration of eyes in cave dwellers, and so on. Obviously, the type of character one should be looking at is one that can be induced in cells which are able to contribute to gametes. In many animals this means looking at characters induced either early in development, or in the germ-line itself. Characters acquired in somatic lineages, even when adaptive, cannot be inherited. (We are ignoring the possibility of horizontal gene transfer from the soma to germ-line in the manner suggested by Steele, 1979.) (v) The adaptations being studied have not always been adaptations to stimuli to which individual organisms *could* adapt. For example, physiological adaptation to high concentrations of DDT is impossible. Only organisms which already have "pre-adapted" mutations can survive such conditions. It has recently been realized that overlooking this simple consideration led to the misleading generalization that all mutations in bacteria are random (Cairns *et al.*, 1988).

There is no doubt that epigenetic inheritance occurs, but what is the importance of epigenetic inheritance? One selective advantage of epigenetic inheritance in unicellular organisms, where EISs presumably first evolved, is probably associated with the relationship between predictable environmental fluctuations and the generation time of the organism. If environmental fluctuations are regular and are longer than the time between two consecutive cell divisions, then individuals who can transmit acquired adaptive functional states to their progeny may be at a selective advantage. Transmitting the acquired state is particularly advantageous when there is a substantial lag between the occurrence of the stimulus and the phenotypic response, because the progeny can respond without going through the induction processes. For example, if an environmental cycle is ten times longer than the generation time, and the probability of induction by that environment is high (nearly 100%), it is

advantageous to have a system which transmits the induced adaptive state to the progeny for about ten generations. Genes that respond to different environmental cycles will have different epigenetic transmission fidelities, each gene having a fidelity which reflects the length of the cycle to which it responds.

Even if the environment itself does not induce a change of state, environmental periodicity relative to the generation time will influence the rate of genetic or epigenetic change from one state to another. Natural selection will determine the average number of generations in each state. It can be shown that for a given periodicity and selection pressure, there is a "best" rate of change (Lachmann *et al.*, in preparation). For example, if the periodicity is 50 times the generation time, and selection against organisms in the wrong state is 10%, then the best rate of change is 5×10^{-2} . This is much higher than normal DNA mutation rates. It suggests that epigenetic systems, rather than the genetic system, have to be used for this type of adaptation to changing environments.

We believe that it was the evolution of epigenetic inheritance systems in unicellular organisms that made the evolution of multicellularity possible. In a multicellular organism, the component cells multiply and die, yet the whole organism retains its coherence and individuality. The maintenance of coherence in the face of constant turnover means that the newly produced components must be similar to the old. This in turn means that some kind of transmission of old states must occur—that an inheritance system which transcends the life span of its component parts operates. The evolution of EISs allowed the development of functional units which could survive longer than the parts from which they were built. If the original unit was an individual cell, the new one is the cell lineage; the functional state no longer ends at cell division, but is more enduring, and encompasses a lineage of cells. Jollos (1921) suggested that the term "individual" need not be restricted to a single paramecium, but could be applied to a population of paramecia, integrated by its response to a stimulus, and delimited by the duration of the Dauermodifikation—by the length of the epigenetic memory of the response—which usually persists from one meiosis to the next. EISs prescribe new time schedules which may transcend those of the individual cell, and allow the emergence of a new unit of function, which is also a new unit of evolution. Without EISs, complex multicellular organisms could not have evolved.

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