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The Germinal Inheritance of Epigenetic Information in Plants

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The germinal inheritance of epigenetic information in plants

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SUMMARY

Germinally transmissible variants that arise by the anomalous imposition of developmental information on the genome are not uncommon in plant genetics, although they are often ignored. Better understanding of such variants is believed to be important because they appear to reflect basic features of developmental control processes. This paper briefly reviews classical genetic studies of such variants in plants, then discusses recent work on the genetic behaviour of plant transgenes, the results of which parallel and extend the classical genetic studies of these phenomena. An attempt is made to explain the molecular basis of these phenomena in terms of modern hypotheses on the dynamic organization of chromatin.

1. INTRODUCTION

The classical view of spontaneous mutation is that it is an undirected process yielding mutant alleles that are stable and qualitatively different from their parent alleles. While this view accurately describes most genetic variants, there are also genetic variants that arise in directed fashion, i.e. by an imposition of epigenetic (developmental) information on the germ line. The term epiallele has been suggested to refer to each different form of a gene that arises in this fashion (Kermicle 1978); Holliday (1987) has suggested a similar term, epimutation, to refer to genetic variants due to DNA modifications. In this paper the definitions of these terms will be modified somewhat so that both refer to the same set of variants†.

Besides being transmissible through the germ line, epialleles are often reversible and epimutable, i.e. they change into new, distinct epialleles. Thus, the process of epimutation can be either cyclical or progressive. Epialleles that exhibit progressive change sometimes produce a continuously varying series of new epialleles which continue to be epimutable. Some epialleles are now known to be correlated with DNA modifications such as cytosine methylation, but whether this is a cause or an effect of epiallelism is still not known (see Selker (1990*a*) for discussion of this problem).

The study of epiallelism in plants has a long and distinguished history as a result of pioneering genetic studies begun in the early 1950s by Brink, McClintock, and Peterson, and followed later by Coe and Kermicle, utilizing maize kernel pigmentation genes. Important early summaries of this body of work are: Brink (1960), McClintock (1965), Peterson (1966), Coe (1966), and Kermicle (1978). By investigating the manner in which new epialleles arise, these workers discovered that three basic assumptions of classical Mendelian genetics are violable. These assumptions are: (i) that in germ line cells the hereditary substance is unaltered genetically by developmental processes; (ii) that following the passage of two alleles through the same nucleus for one heterozygous generation, each allele emerges unaltered in segregating progeny; and (iii) that gene expression following passage of a gene through a male gametophyte is equivalent to gene expression following passage of the same gene through a female gametophyte. The first assumption was found to be violated in the phenomenon of activity cycling in transposable elements, the second in the phenomenon of paramutation, and the third in the phenomenon of parental imprinting. Each of these will be briefly summarized below.

Because epimutation is a directed process, biologists have long recognized that it might reflect fundamental mechanisms of plant developmental control, perhaps for the establishment and maintenance of stable developmental states; and epiallelism has been invoked to explain processes as varied as the transitions from juvenility to adult vegetative and adult reproductive phases, the elaboration of pigmentation

† Because of the close similarity between the terms epiallele and epimutation, as well as the overlap in their definitions, it would be difficult to use both terms without modifying them to be consistent with one another. For the purposes of this paper, then, both terms will be used to refer to the same set of genetic variants, inclusive of both DNA modifications and developmentally imposed genetic variants. This is a broad definition, but the lack of precise information regarding the molecular cause of most heritable epigenetic variants, coupled with the benefits of consistent terminology makes this broad definition preferable for now. The term epimutation will be used also to refer to the process by which epialleles and epimutations arise, and epimutability is a property of any gene that is subject to the process of epimutation. Genomic imprinting is a broader term that includes germinally heritable epigenetic variants, but also refers to events that are somatically heritable, but not germinally heritable. It has been applied mostly in animal systems (Monk & Surani 1990), but also has use in plant systems (Federoff *et al.* 1989; Matzke & Matzke 1993).

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patterns, and cytokinin habituation (Brink 1962; Poethig 1990; McClintock 1967*b*; Fedoroff *et al.* 1989; Meins 1989).

2. CYCLIC PHASES OF ACTIVITY IN TRANSPOSABLE ELEMENTS

McClintock discovered cycling epialleles of maize transposable elements (TEs). She referred to these as changes in phase of the TEs to distinguish them from stable, undirected mutations in TEs (which she called changes in state and have been shown to be caused by DNA sequence alterations (reviewed by Fedoroff *et al.* (1989)). Among studies of maize TEs exhibiting cyclical changes in phase (reviewed by Fedoroff (1983); see also Martiennsen *et al.* (1990)), McClintock's and Peterson's studies of the *Spm* element (also known as *En*) provide the most thorough understanding of this phenomenon.

Phases of *Spm* activity can be monitored visually and non-invasively by observing their effects on, and control over, an anthocyanin pigmentation gene into which the element (or its non-autonomous derivative) is inserted. Although different phases of activity of the *Spm* element are heritable through meiosis, they are also reversible and highly changeable. Different phases of *Spm* exist in which the element is active, inactive, or exhibiting intermediate expression. Among phases with intermediate expression, McClintock and Peterson described a very diverse array of phases that differ complexly with respect to the pattern of pigment distribution throughout the plant.

An especially significant observation is that the epimutability of these different epialleles of the *Spm* element varies from one to the next and that epimutation is not stochastic, but is dependent on developmental context. Another important feature of *Spm*'s phase change behaviour is the fact that an active *Spm* element can preset certain responsive, non-autonomous *Spm* elements for a new expression pattern (phase) which will be expressed at a later time in development, even in the absence of the element that causes the presetting. This setting typically is erased during sexual reproduction.

With regard to the mechanism behind the cycling phenomenon, McClintock (1967*a*) proposed that the phase of an element has both a consequence and a cause relating to normal mechanisms controlling gene expression. The proposed consequence of a particular phase is that it may affect the positional (or functional) domains in which the element itself will be located in the nucleus of particular cell types, resulting in developmentally varying expression patterns. Reciprocally, certain nuclear domains may cause a change in phase through the imposition of developmental information on the element. Thus, as McClintock (1967*a*) suggested 'a relatively simple sequence of events' involving reciprocal influences between genes and domain-specific nuclear features might lead to a complex series of expression patterns. Technologies for determining the locations of genes in the nuclei of different cells in order to detect such repositioning of genes are becoming available in some systems (e.g. Manuelidis 1990), offering the hope that this part of McClintock's suggestion for *Spm*'s phases can be tested in the near future.

The molecular basis for epimutations in several maize TEs, including the *Spm* element, has been explored by monitoring the methylation of cytosines with methylation-sensitive restriction enzymes. The first published demonstration of a correlation between methylation and inactivation of a TE was by Bennetzen (1985), and similar correlations have been found in several other systems since (summarized by Dennis & Brettell 1990; Martienssen *et al.* 1990). In the case of *Spm*, the pattern of methylated cytosines appears to vary in concert with changes in the element's phase of activity, both developmentally and germinally (Banks & Fedoroff 1989), suggesting that methylation pattern is a result, or maybe a component, of the cell-type-specific domains of the nucleus predicted by McClintock.

3. PARAMUTATION

The phenomenon of paramutation is as remarkable as is the cycling and presetting of activity phases of transposable elements. Paramutation results from the action of particular alleles possessing paramutagenic properties on certain other alleles with paramutable properties. The outcome is the alteration of the paramutable allele into a new paramutant allele whose phenotypic expression is germinally heritable, and is expressed even in the absence of the paramutagenic allele. In other words, paramutation is an interallelic interaction resulting in the epimutation of one allele as a result of the presence of the other. Although paramutation seems to have been observed in plants almost since the beginning of genetics, it was generally overlooked until R.A. Brink (1960) carefully reconsidered and defined, and then publicized the phenomenon. Paramutation is often considered to be enigmatic because it is an apparent violation of one of the basic tenets of genetics: that allelic genes, though they may affect one another's expression when present in the same nucleus, emerge unaltered upon segregation in sexual progeny. (For a recent review of paramutation, see Dooner *et al.* (1991).)

Paramutation at the maize *R* locus exhibits unusual patterns of instability. Paramutants of *R* appear progressively in development and change progressively and heritably over successive sexual generations, either decreasing in expression in the presence of a paramutagenic allele, or increasing (gradually returning to maximal, stable expression) in its absence. Many *R* paramutants become relatively stable in homozygous condition. Thus, like epimutation, paramutation of *R* exhibits reversibility and heritability, and is also progressive and quantitative. On the basis of these unusual properties, Brink speculated that the mechanism behind paramutation would involve changes not in the DNA, but in the organization of the chromatin encasing the affected genes. This led him to propose the concept of a paragenetic function for chromatin, as well as the term parachromatin for the type of chromatin fulfilling that function

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(Brink 1960). The DNA methylation state of genes involved in paramutation has not yet been reported in the literature, although unpublished data indicate a correlation between methylation and paramutation at the *R* locus (M. Alleman & J. L. Kermicle, personal communication).

The presetting of phases of *Spm* somewhat resembles paramutation. Presetting alters the element with respect to its expression at a later time in development and does not require the presence of the other element at the time of expression. However, unlike paramutation, the setting usually is erased during sexual reproduction. Exceptions in which erasure of the setting do not occur are analogous to paramutation with the paramutagenic agent being an active *Spm* element, which can be either allelic or non-allelic to the 'paramutable' preset element. McClintock's interest focused on the erasure process because it indicated that presetting was subject to a developmental rule that also applied to any epigenetic process in normal development, i.e. reversal during the life cycle of the plant. In contrast, Brink's interest was focussed on the heritability of epigenetic changes.

4. PARENTAL IMPRINTING

Parental imprinting was first described in animals for the differential silencing or elimination of maternal and paternal chromosomes. Through further investigation of paramutant *R* alleles in maize, Kermicle discovered the first example in eucaryotes of parental imprinting of a single gene (Kermicle 1978). This effect was limited to the endosperm tissue of the maize kernel, the triploid product of a fertilization event separate from that producing the embryo. Thus, no grandparental imprinting effects are observed. The lack of imprinting in embryonic tissues (which are the result of a separate fertilization event in maize) indicates the precise control over imprinting that is exerted by the organism (Kermicle & Alleman 1990). Genetic variation for the control of imprinting also exists in maize at an unlinked modifier locus.

5. TRANSGENE EPIMUTATION

Transgenes offer an additional opportunity to study epimutation phenomena in plants. The ability to vary the chromosomal location and sequence context of a gene could have a significant effect on our understanding of, and control over the factors involved. Although it is not yet possible to manipulate sequences in the plant genome at will (homologous recombination technologies that have practical application have yet to be developed in plant systems), there is already considerable information available on epimutation of transgenes.

The clearest demonstration of transgene epimutation in plants is provided by the use of genes for anthocyanin pigmentation. Because maize transformation–regeneration systems have been developed only recently, the available information on pigment transgenes has been obtained mainly in petunia flowers. The best example involves a maize anthocyanin gene that was used to complement a pigmentation defect in an anthocyaninless petunia line (Meyer *et al.* 1992). It was found that the expression of the (single copy) transgene, though initially judged to be stable in the primary transformant and in a homozygous line derived from it, was subject to various types of epimutation. Thirty thousand hemizygous progeny of a single homozygous plant were scored for flower colour, 11 000 of which were monitored throughout an entire growing season, and the following observations were reported.

(1) A single classical mutation was uncovered, a small deletion within the coding sequence of the transgene.

(2) Plants producing variegated (red sectors arising on a white background) flowers appeared at a frequency of 2×10^{-3}. None of these resulted from the insertion of transposable elements, or from any other DNA rearrangement. Rather, all variegated plants appeared to be the result of epimutation because each showed hypermethylation of the promoter. Red sectors were inferred to be the result of somatic reversion of the white, epimutant phenotype. Further genetic analysis of these plants is required to determine the heritability and stability of these phenotypes.

(3) Most plants in the population showed weak or no transgene expression, and this correlated with methylation of the transgene promoter.

(4) 0.3% of those plants monitored throughout the growing season exhibited somatic loss of expression of the transgene (white sectors on a red background), again correlated with promoter methylation.

(5) The phenotypic expression and promoter methylation of the transgene were both subject to environmental and developmental influences. Interestingly, it could be shown that the age of the parent plant determined the susceptibility of transgene expression to environmental influences in the next generation, indicating that heritable alterations tend to occur late in development. Parallels with the epimutation behaviour of maize transposable elements are obvious. Clearly, then, pigment transgenes can be useful for investigating epimutations in plants and broadening the base of information on this process.

The fact that a heterologous pigment transgene exhibits a high degree of epimutability, while the endogenous petunia pigment genes generally do not, suggests that it is the sequence context of the transgene that determines its epimutability, as is proposed for animal transgenes. In maize, transposable element insertions into anthocyanin genes were the basis of epimutability, which in any case was limited to the TE. On the other hand, paramutation at the *R* and *B* loci in maize does not involve TE insertions, as far as is known, but might depend on particular sequence organization patterns. Structural analysis of different alleles of the *R* and *B* loci might illuminate this question, and the analysis of *R* and *B* transgenes in maize also might be very informative.

6. INTERLOCUS AND INTERALLELIC TRANSGENE INTERACTIONS

Matzke *et al.* (1989) discovered interactions between

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unlinked transgene loci which shared some stretches of homology with each other, resulting in one locus being inactivated and methylated in the presence of the other. Both the inactive state and the associated methylation of the first locus are reversible in the absence of the second locus, but this reversion is gradual, occurring over several generations (Matzke & Matzke 1990, 1991). This paramutation-like interaction between unlinked transgenes has been observed with another pair of transgenes by Goring *et al.* (1991), although no methylation was detected in that case. Paramutation resulting from an interaction between alleles of a transgene locus differing in sequence organization has been demonstrated by J. English and J. Jones (personal communication). Also, epialleles of a transgene locus can interact to result in the apparent paramutation of one epiallele by another (R. Jorgensen, unpublished data). These observations of paramutation in transgene–transgene interactions would appear to rule out the possibility that a specialized protein encoded by the paramutagenic locus (perhaps analogous to the SD product involved in meiotic drive in *Drosophila*) of the paramutagenic gene is responsible for paramutation, as might be possible for endogenous paramutagenic genes.

Interlocus effects are not limited to transgene–transgene interactions, as there are now many examples whereby a transgene can cause the suppression of a homologous, endogenous plant gene (see, for example, Napoli *et al.* (1990); van der Krol *et al.* (1990); Smith *et al.* (1990)). An intriguing feature of transgene–endogenous gene interactions is that commonly the expression of both genes is suppressed (referred to as co-suppression), even though the genes are driven by unrelated promoters. However, it is not known whether transgenes suppress homologous, endogenous genes via the same mechanism as transgenes suppress other, homologous transgenes. In fact, no paramutation-like suppression of an endogenous gene after segregation of the homologous transgene has been reported. The available data on *trans*-inactivation by transgenes, along with several hypotheses attempting to explain them, have been reviewed recently (Jorgensen 1992).

7. EPIALLELIC VARIATION IN ORGANIZED FLOWER PIGMENTATION PATTERNS IN TRANSGENIC PETUNIAS

Interference with the expression of an endogenous plant gene by a homologous transgene can be surprisingly complex. This has been illustrated best with flower colour genes, especially the chalcone synthase gene (Napoli *et al.* 1990; van der Krol *et al.* 1990; R. Jorgensen, unpublished data). Chalcone synthase is involved in the biosynthesis of anthocyanin pigments, and co-suppression of an endogenous chalcone synthase gene and the transgene results in the production of white flowers or flowers with developmentally determined colour patterns. The flower pigmentation patterns are comprised of white sectors which are caused by the co-suppression of the expression of the transgene and the endogenous chalcone synthase gene. These patterns are highly organized and appear to be determined by cells located either at the edges of petals or in or near the vasculature of petals, and

Figure 1. Suppression of anthocyanin pigmentation by chalcone synthase transgene. Five petunia flowers representative of non-clonal patterns produced by derivatives of a single transgenic plant are shown. For contrast, flower in upper right illustrates the shape of typical clonal sectors as might be produced through transposable element mutations. Among the five non-clonal patterns, at least three genetically distinct derivatives of a single transformant are represented by the flowers at the upper left, lower left, and lower right. The lower middle flower is genetically similar to the one at the lower right. The upper middle flower is genetically similar to the upper left.

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possibly by cells underlying the epidermal cells which elaborate the pigmentation pattern (figure 1). The white sectors of each pattern are non-clonal and have diffuse boundaries, that is, the sectors are many cells wide. Furthermore, pattern boundaries are coincident in the upper and lower epidermis. Thus, elaboration of these patterns must require intercellular communication. Moreover, patterns appear to be determined similarly to (and perhaps based on) morphological patterns that determine petal shape.

What is interesting in the context of this paper is that a complex series of somatic and germinal variants appear spontaneously in these transgenic petunias. Such variants display new patterns different in form from the parental pattern. Examples are shown in figure 1. The frequency of variants is especially high following adventitious shoot regeneration from tissue explants (a procedure capable of altering methylation patterns). Many of these variants are germinally heritable, and they are reversible somatically as well as germinally, i.e. they are epialleles. DNA rearrangements have not been ruled out yet; nor is it clear whether these epialleles map to the transgene locus or the two endogenous CHS loci (CHS A&J) or another locus.

Perhaps most significant is the observation that the occurrence of epimutations can be non-random, with bursts of independent epimutations arising at the same time in mature plants. Thus, not only are these epimutations reversible, heritable and changeable, but they occur in response to developmental circumstances. Some somatically occurring epialleles are not inherited by all progeny, but are 'reset' to the original epiallelic state during sexual reproduction or in early development. In sum, this transgenic system displays the same features that McClintock described for Te activity cycling. It also illustrates the complexity and lability of gene expression patterns that can be produced by transgenes.

8. THE PARAGENETIC FUNCTION OF THE GENOME

The remainder of this paper will discuss various concepts that might be useful in understanding the process of epiallelism in plants. Earlier I described McClintock's concept of reciprocal influences between genes and domain-specific nuclear features. A related and somewhat more detailed concept came from R. A. Brink (1960, 1962) who proposed that the genome serves a paragenetic function in addition to its genetic one, the former being concerned with the development of the individual, the latter with inheritance. In Brink's words, the chromosomes not only 'carry an unchanging complement of genes in somatic cells, but also possess other, more labile components by which gene action is regulated during development'. He further suggested that a particular paragenetic state is self-maintaining in mitosis, but subject to influences from the developmental and physiological environment within the organism, i.e. it is developmentally paramutable. Thus, 'the individuality of a somatic cell is implicit in the paragenetic state of its chromosomes'.

Brink proposed the term parachromatin to refer to whatever chromosomal features are responsible for the paragenetic state. It is important to recognize that the concept of parachromatin was not meant to be limited to genes which are germinally paramutable. Rather, Brink regarded germinally transmissible paramutants only as aberrant manifestations of a fundamental process that might be relevant to the behaviour of all plant genes, and perhaps all higher eucaryotic genomes.

9. GENOMIC IMPRINTING AND CHROMATIN ARCHITECTURE

Brink's concept of parachromatin anticipated to a considerable extent the modern concept of genomic imprinting. Genomic imprinting is a term introduced by animal biologists to refer to the clonal inheritance of developmentally imposed states, as is apparent in parental imprinting and X-chromosome inactivation in mammals, and in position effect variegation and polycomb gene behaviour in *Drosophila* (for examples, see reviews by Surani (1991); Monk (1990); Henikoff (1990); Paro (1990)). Among these phenomena only parental imprinting shows any germinal inheritance, and parental imprints are generally erased each generation to be replaced by a new imprint. How this concept is applicable to epiallelism phenomena in plants has been discussed in some detail by Matzke & Matzke (1993).

Since 1960 when Brink proposed the parachromatin concept, much has been learned about the components of chromatin from both genetic and molecular studies of genomic imprinting phenomena. These studies have led to the suggestion that it is 'the architecture of chromatin, in contrast to the regulatory networks of diffusible factors', that serves 'as a cellular memory transmitting the determined state of a cell to its clonal descendants' (Paro 1990). Investigations of the complexity, dynamics and stability of multi-component DNA-protein complexes (i.e. chromatin) at specific loci are now possible for some well-characterized genes. These biochemical studies now suggest at least two types of mechanisms by which chromatin structure may be altered developmentally: dynamic competition and pre-emptive competition, which differ primarily in the stability (or resistance) of chromatin to potential disruption by newly synthesized factors (Felsenfeld 1992). Similarly, genetic and molecular studies of *Drosophila polycomb* genes and *suppressor of position effect variegation* genes indicate that clonally propagated states involve heterochromatin proteins (Paro 1990). Thus, chromatin architecture could be the basis for a stable, yet flexible, ontogenetic cell memory.

Recently, greater resolution of chromatin organization has been achieved by the discovery and characterization of a class of chromatin components known as 'global activators'. These have been best characterized in yeast but found to be widespread and highly conserved among eucaryotes. These global factors interact with both gene-specific transcription factors and basic chromatin components such as histones and

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HMG proteins. One of the functions of global activators is a helicase activity. It has been suggested that the primary role of the helicase function is to reprogram chromatin and to establish both repressed and active states of chromatin (Travers 1992; Winston & Carlson 1992).

10. THE ORIGIN OF EPIALLELES AND GENE IMPRINTS

What then might be the origin of epigenetic variants that have sufficient stability as to persist through germinal transmission? From the perspective outlined above, it is conceivable that certain multimeric chromatin structures could be perturbed through developmental fluctuations in the levels or modifications of transcription factors or other chromatin components. These perturbations might lead in turn to alterations in DNA methylation patterns due to exposure or protection of DNA. DNA methylation provides an attractive mechanism for the inheritance of paragenetic information because DNA methylation is known to be heritable in some cases. Mechanistically this heritability can be explained by the fact that replication of DNA produces hemi-methylated DNA which is then recognized by a maintenance methylase to preserve the methylation pattern (Holliday 1987). Assuming that DNA methylation patterns are sufficient to maintain local chromatin organization patterns through multiple generations of cell division, it is conceivable that methylation could stabilize a developmentally instigated perturbation of chromosome organization, and also that interference with the maintenance methylation process is one possible cause of epimutations.

It is also possible that DNA methylation is not always sufficient, or even necessary for heritability of epimutations, and that other mechanisms, such as higher order levels of chromosome organization, might be responsible for the inheritance of 'paragenetic' information (Riggs & Pfeiffer 1992). Thus, it may also be necessary to look beyond the local aspects of chromatin organization in order to understand epiallelism. The types of chromosomal domains that might be responsible for the paragenetic state of a gene could be functional, spatial or temporal. Functional differences between alternative paragenetic states (i.e. epialleles) of a given gene could involve DNA methylation as well as the presence, the concentration and the modification state of certain chromatin components. Spatially distinct alternative states could arise via movement of a chromosome segment between different nuclear locations capable of conferring different gene expression potentials. Temporal differentiation of 'parachromatin' could be achieved by modifying the time of DNA replication in the cell cycle, late replication being commonly associated with lack of transcription, as has been discussed by Riggs & Pfeiffer (1992).

It is important to address the question of why some genes are epimutable and most are not. Assuming that the stability and flexibility (i.e. epimutability) of the alternative chromatin structures at a locus depends to some degree on the DNA sequence context of and around the gene, it would be expected that the sequence context of most genes would have been selected by evolution to resist any imposition of germinally heritable alterations during ontogeny. Occasionally new alleles will arise that fail to resist such forces, i.e. alleles that are epimutable or paramutable. Although these may not survive in evolutionary time, they would be observed as unstable variants by plant breeders and geneticists. The fact that the most closely studied examples of epiallelism in plants involve kernel and plant pigmentation in maize may not be coincidental. The well-known cultural importance of pigmented maize lines to the indigenous peoples of the Americas may be responsible for much of the genetic diversity behind this phenotype that is available to modern geneticists. In the case of plant transgene loci, epimutability, paramutability, and paramutagenicity might be unusually common due to the abnormal sequence context in which they often may lie.

(Note: As was indicated in the footnote on the breadth of the definition of the terms epiallele and epimutation, DNA rearrangements are not excluded as possible causes of these phenomena. DNA rearrangements that could cause epimutations of this broad definition would have to be of a type that are reversible or highly unstable. An example would be phase variation in *Salmonella typhimurium* or mating type determination in *Saccharomyces cerevisiae*. In the case of TES, these elements are capable of mediating transposition, inversion, and deletion events that can generate a diverse series of new alleles which are typically stable and irreversible. Events such as these are known to be responsible for changes in state, i.e. classical mutations of the TE, as well as mutations of adjacent genes (Federoff 1983). McClintock distinguished changes in phase from changes in state by the cyclical behaviour of the former. It is now known that changes in phase of TES do not involve DNA sequence alterations, but are correlated with DNA methylation (Banks & Fedoroff 1989). Nevertheless, it is important to note the possibility that reversible sequence rearrangements could produce the same phenomenology as changes in phase and meet Kermicle's definition of epiallele. In fact, the occurrence of such rearrangements could be responsive to developmental or environmental influences because the expression of transposase can be developmentally controlled. Thus, it is essential that the investigation of the mechanisms behind epiallelism test for the involvement of DNA rearrangements.)

11. MECHANISMS BEHIND INTERALLELIC AND INTERLOCUS INTERACTIONS

(*a*) *Chromatin perturbation and competition for diffusible factors*

It is conceivable that one gene (or allele) could affect another through competition for diffusible factors. For example, if two genes differ in their ability to compete for such factors, one might perturb the other's

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chromatin organization. Or, in the case that a gene's interaction with a factor is dependent on a certain threshold level of that factor, introduction of additional gene copies could reduce the amount of the factor that is available to each gene copy below the needed threshold level. Because most transgene loci do not exhibit an interlocus *trans*-interaction, Matzke & Matzke (1990) suggest that any such diffusible factors may be diffusion-limited such that the relative nuclear locations of two genes would determine whether any *trans*-interaction could occur. A difficulty with this model is that it does not explain the specificity, i.e. why no other genes appear to paramutated.

(*b*) *Homology-based interactions between nucleic acids*

Recent discoveries in fungal and animal genetics suggest two additional hypotheses for how paramutation might occur. Both hypotheses propose that nucleic acids might interact with the paramutable gene in a homology-dependent manner, and that the result of this interaction could be a perturbation of chromatin organization or DNA methylation pattern. These hypotheses and their implications have been described in a recent review (Jorgensen 1992) and so they will be summarized here only briefly.

The first possibility is that transcripts from a paramutagenic gene might form an RNA–DNA hybrid (perhaps triple-stranded) with the paramutable gene (also considered by van der Krol *et al.* (1990) for transgene-caused gene silencing). Such an RNA might be either a sense or an antisense strand. It is at least conceivable that RNA base-pairing with a gene *in trans* could perturb chromatin structure and so cause its paramutation. Such a mechanism remains to be demonstrated in plants and remains purely speculative for now. So-called 'anti-gene' RNAs are the subject of serious attempts to attenuate animal gene expression (reviewed by Helene (1991)).

The second possibility for nucleic acid interactions is that homologous genes might pair directly with one another. Such pairing might be either allelic or ectopic (non-allelic). Very efficient ectopic pairing is known to occur in the RIP system in *Neurospora*, as well as in ectopic gene conversion in yeast and *Drosophila*. The filamentous fungus *Neurospora crassa* exhibits a phenomenon resembling the transgene-caused gene silencing phenomenology of plants that is called repeat-induced point mutation (RIP) whereby any duplicated sequence becomes mutated and methylated at a premeiotic stage of its reproductive phase. This process appears to require pairing of homologous sequences followed by recognition of the pair by a system for DNA and chromatin modification (Selker 1990*b*). Analogies have been drawn between RIP and ectopic gene conversion in yeast (Haber *et al.* 1991). Unlike transgene-caused gene silencing in plants, RIP occurs only rarely, if at all, in somatic diploid fungal cells. Evidence of a 'slow RIPping' process in animals comes from the comparison of rates of C-to-T mutations in multi-copy genes as compared to single-copy genes. Considering that these mutations must have occurred in the germ-line, Drake (1991) has suggested that both 'slow ripping' in animals and 'fast ripping' in fungi are limited to the haploid phase of the life cycle.

For paramutation to occur as a result of any nucleic acid interactions, chromatin organization and DNA methylation properties of the target gene must be susceptible to perturbation as a result of these interactions. Thus, whether a persistent alteration in chromatin organization or DNA methylation pattern might occur would depend on the susceptibility of the target gene, as well as on the paramutagenic features of the trans-acting RNA or DNA molecule. That most genes fail to exhibit paramutation in plants is presumed to be a reflection of the fact that natural selection generally would eliminate paramutable alleles, i.e. those whose chromatin organization is not reset during sexual reproduction. This is consistent with Brink's view of paramutation as an aberrant process reflecting the existence of certain normal features of chromatin organization or dynamics.

(*c*) *Plants contrasted with animals*

It is intriguing that transgene-caused silencing of homologous genes has not yet been described for transgenic animals. Unless this reflects the possibility that such effects have gone unnoticed in animals, it would seem that plants possess features of gene regulation or nuclear organization. On the other hand, Monk (1990) and Tartof & Henikoff (1991) have speculated that animals may have 'cross-talk' or 'trans-sensing' mechanisms operating in somatic cells for communication between homologues with regard to the epigenetic state and history of genes. If so, then perhaps the main difference between plants and animals in this regard is only a matter of degree.

It is interesting also that no true parallel to paramutation has been revealed by animal genetics. Brink (1960, 1973) cites several possible examples of paramutation in *Drosophila* (meiotic drive and unequal crossing-over within arrays of tandem repeats); however, these would now appear to be unrelated mechanistically to paramutation. This apparent difference between plants and animals might be explained if animals restrict both the creation and the erasure of epialleles to specific developmental stages. In animals most epialleles arise through parental imprinting, i.e. during sexual reproduction (see Surani *et al.*, this symposium). The highly efficient parental imprinting in animals ensures the erasure of epialleles arising in the grandparental generation. Other than the gametic imprinting of endosperm genes, the closest parallel in plants is the efficient erasure of presetting.

Without denying the broad similarities in gene regulatory mechanisms and chromatin components known to be shared by plants and animals, it is nonetheless reasonable to ask whether their nuclei might differ significantly with respect to nuclear or chromosome organization patterns, especially in terms of higher order structures, about which little is yet known. Certainly, it is true that these phyla have been

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evolutionarily distinct for a very long time, having diverged prior to each kingdom's independent evolution of multicellularity and of a prolonged, specialized diploid phase in their life cycles. Thus, these phyla have had ample opportunity to evolve significant specializations in the dynamic organization of chromatin and nuclei.

12. IMPLICATIONS FOR THE ORIGIN OF DIRECTED MUTATIONS

The occurrence of selection-induced mutation processes yielding irreversible alterations in DNA sequence has been reported repeatedly in bacteria and recently in yeast (Hall 1992). The directed nature of the process of epimutation is intriguing in this regard. With regard to the origin of selection-induced mutations, one could speculate that environmental factors could induce epiallelic variants in normal genes, and that such variants, even if unstable, might predispose the gene to be either more or less sensitive to mutagenic molecular machinery, altering the rate of mutation at the locus. If a higher rate of mutation occurs, a DNA sequence variant might arise that stabilizes the new epiallelic state of the locus. It is conceivable that only those DNA alterations which in fact stabilize the epiallele would be permitted or favored.

A specific proposal of this sort has been proposed by Flavell (1991). It is based on rRNA genes in plants and the knowledge that: (i) not all rRNA genes with the same function are in the same physical state in the nucleus; (ii) these states differ with respect to their accessibility to transcription machinery; and (iii) changes in the number of tandem copies of rRNA genes are subject to phenotypic selection. Thus, it is necessary to postulate only that recombination enzymes are differentially able to produce genetic variation in different rRNA gene loci. Though less information is currently available on the nature of epialleles than on the nature of states of rRNA gene loci, the possibility that epiallelism could be related to the selection-induced mutation phenomenon is at least worth considering because the implications for evolutionary biology could be significant (see, in addition, Monk (1990)).

13. A CONCLUDING PERSPECTIVE

After consideration of the various exceptions to Mendelian principles reviewed here, it is difficult to escape the conclusion that science is still extremely ignorant about the functioning and organization of the nucleus. In spite of the remarkable advances in molecular dissection of many nuclear processes, we still can not say we really understand gene expression and control. And in spite of the powerful appeal of the principles of population genetics for explaining the prime forces in the evolution of species, the parallel phenomena of directed epimutation and selection-induced mutation suggest that perhaps we understand far less about these forces than most scientists had recently assumed. Thus, nearly a century after the rediscovery of Mendel's laws, the challenge to understand the basis for the remarkable diversity of known genetic phenomena in terms of cellular organization and function remains nearly as great as ever.

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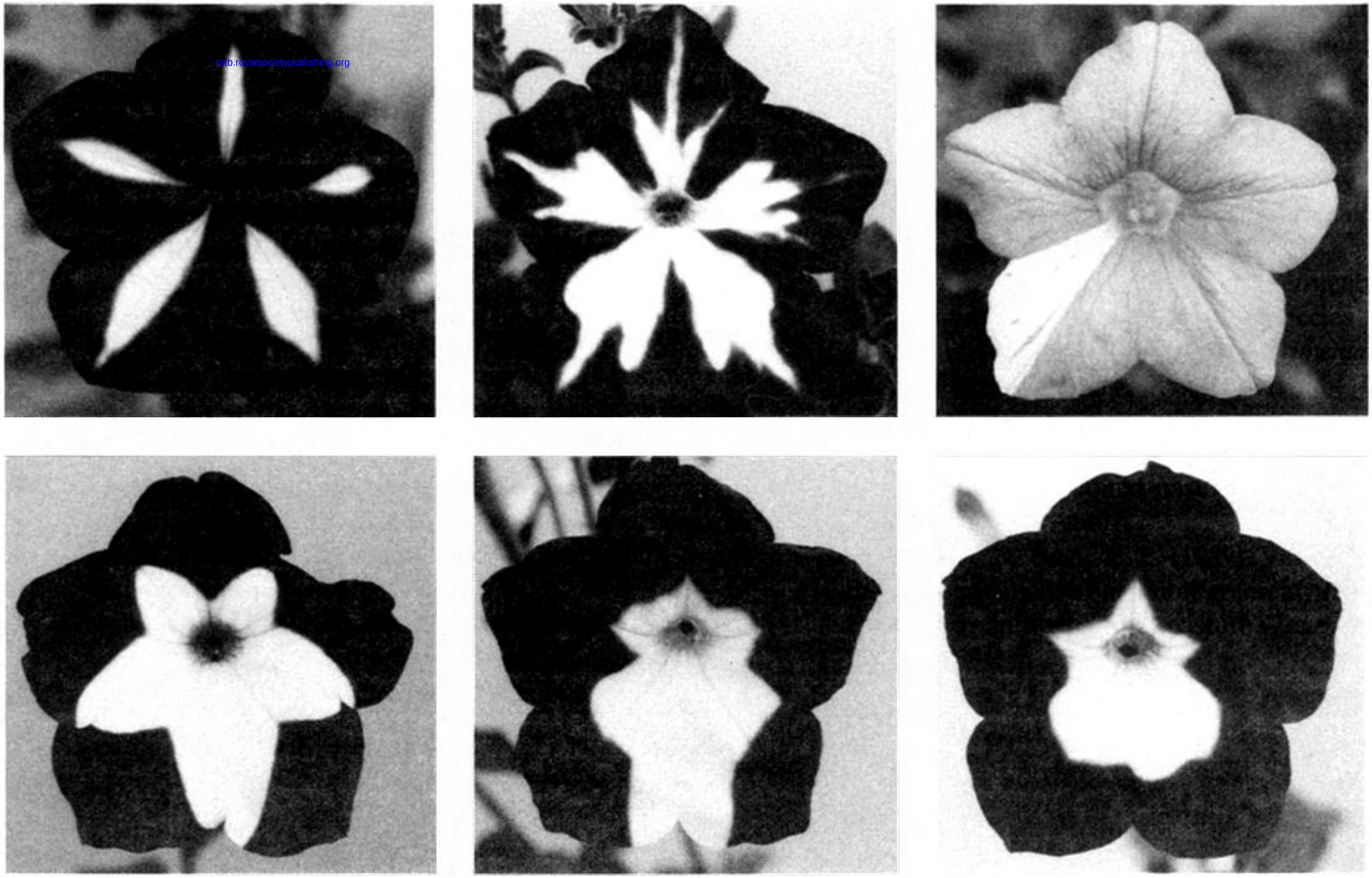

igure 1. Suppression of anthocyanin pigmentation by chalcone synthase transgene. Five petunia flowers presentative of non-clonal patterns produced by derivatives of a single transgenic plant are shown. For contrast, ower in upper right illustrates the shape of typical clonal sectors as might be produced through transposable ement mutations. Among the five non-clonal patterns, at least three genetically distinct derivatives of a single ansformant are represented by the flowers at the upper left, lower left, and lower right. The lower middle flower is enetically similar to the one at the lower right. The upper middle flower is genetically similar to the upper left.