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Paramutation and transgene silencing: a common response to invasive DNA?

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Some cases of paramutation of endogenous plant genes and silencing of introduced genes suggest the action of a genomic defence system that inactivates and methylates invasive DNA sequences such as transposable elements and multiple copies of transgenes. Paramutation can occur when promoters of repeated endogenous genes contain transposable elements that are highly homologous to other elements in the genome; the endogenous gene is then mistakenly identified as invasive. Transgenes can be recognized as being invasive either during integration or after integration if multiple copies are present. Because transposable elements are often associated with plant genes, the dividing line between endogenous and 'foreign' genes is not always clear cut. The blurring of this distinction could account for the similar epigenetic behaviour of many transgenes and paramutable endogenous genes, and might have broader implications for the regulation of plant gene expression.

When present as multiple copies, introduced and endogenous sequences in transgenic plants can exhibit a variety of epigenetic phenomena that are collectively termed homology-dependent gene silencing[1] or repeat-induced gene silencing[2] ('epigenetic' refers to the information content of the genome that does not reside in the primary nucleotide sequence). Some examples of these phenomena appear to be similar to paramutation, which is an epigenetic process described for several endogenous plant genes. The hallmark of both homology-dependent gene silencing and paramutation is that one allele or locus is able to induce a heritable change, in the form of weakened expression, in a second allele or locus. The reduced activity is often, but not always, associated with increased cytosine methylation. Despite the similarities, it is not known whether a common mechanism underlies both phenomena. The degree to which this mechanism impinges on normal gene expression is also unclear. Here, we review data suggesting that some cases of endogenous gene paramutation and transgene silencing share a common basis in a response to invasive DNA, which effectively neutralizes proliferating sequences by methylation. In this view, transgenes that become silenced are recognized as being invasive because they are present in multiple copies; endogenous genes are

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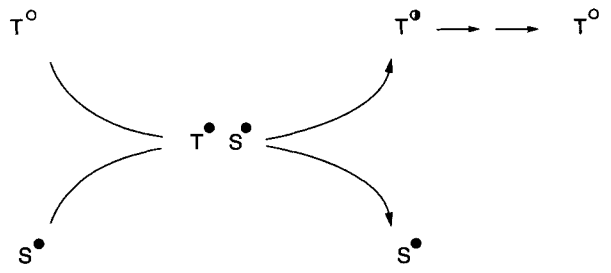

Fig. 1. *Trans*-silencing. A target locus or allele (T), which is usually expressed and not methylated, can become methylated and exhibit reduced activity either during (transgene silencing, as shown here) or following (*r* paramutation, as shown in Fig. 3) an association with a methylated silencing locus or allele (S). The methylation and silencing can persist for a few generations but is gradually reversible in the absence of the silencer, which is unchanged by the interaction. More completely methylated, partially methylated and sparsely methylated states are indicated by filled circles, half-filled circles and open circles, respectively.

incorrectly identified as invasive because they are repetitive, and because their promoter regions contain transposable elements that are highly homologous to other elements in the genome.

DNA methylation as a defence response to invasive sequences

Evolutionary considerations suggest that DNA methylation in eukaryotes derives from a prokaryotic genomic defence system that disables invasive or foreign DNA sequences[3]. The advantages of such a system appear obvious: proliferating sequences introduce the potentially lethal problems of insertional mutagenesis and ectopic recombination that could disrupt chromosome structure. Multiple copies of a particular DNA sequence can arise through replicative transposition of transposable elements or genomic turnover processes (e.g. unequal crossing over). Filamentous fungi possess two alternative ways for dealing with these potentially deleterious sequence duplications.

Fate of duplicated sequences in filamentous fungi: repeat-induced point mutation and methylation induced premeiotically

Transformation experiments have shown that filamentous ascomycete fungi frequently eliminate one copy of a tandem duplication (i.e. where two copies of a gene occur in series) by recombination. *Neurospora crassa* and *Ascobolus immersus* have also taken a 'bolder step'[4], which involves modification of both copies of a duplicated sequence. In *Neurospora* this occurs by C-to-T transitions (RIP, 'repeat-induced point mutation') accompanied by frequent methylation[4], and in *Ascobolus* by methylation (MIP, 'methylation induced premeiotically')[5]. Although RIP involves point mutations, these might actually be the result of aborted methylation attempts under conditions in which the methyl donor, *S*-adenosylmethionine, is limiting[4]. Therefore, it is possible that RIP and MIP represent the same response to DNA duplications. Both RIP and MIP neutralize repeats by sequence diversification (rapidly in RIP, more slowly via spontaneous deamination of 5-methyl-C to T in MIP) and by methylation, which inhibits transcription and probably also prevents recombination[5]. Recently, an attenuated MIP

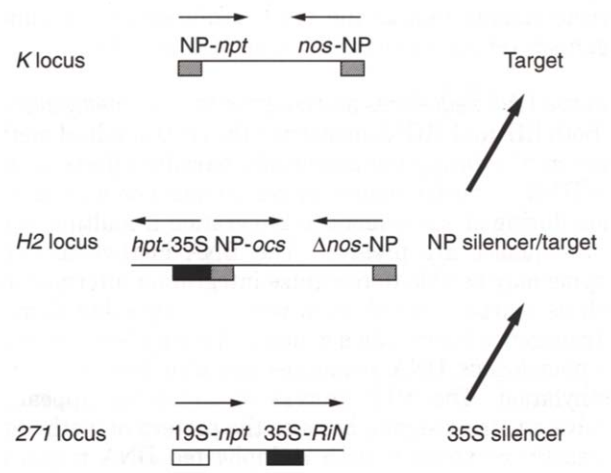

Fig. 2. Transgene constructs present at promoter homology-dependent silencing loci. The nopaline synthase promoter (NP) silencing locus, *H2*, which comprises about four copies of a transgene construct containing two copies of the NP (hatched box), can silence NP-driven genes at unlinked target loci (i.e. the *K* locus[16]). The silencing locus, *271*, which comprises six to seven copies of a transgene construct containing one copy each of the 19S and 35S promoters of cauliflower mosaic virus (19S, open box; 35S, filled box), silences 19S- and 35S-driven genes at unlinked target loci. Interestingly, it appears that the 35S-*hpt* gene at the *H2* locus is the most sensitive target gene yet found to be silenced by the *271* locus[15]. The multiple copies of the NP at *H2* and the 35S at *271* are all highly methylated. Abbreviations: *npt*, neomycin phosphotransferase gene; *nos*, nopaline synthase gene; *hpt*, hygromycin phosphotransferase gene; *ocs*, octopine synthase gene; *RiN*, nitrite reductase cDNA in antisense orientation. The *nos* gene at the *H2* locus is truncated, as indicated by the 'Δ'. Horizontal arrows indicate the orientation of genes in each construct. Heavy diagonal arrows point from a silencing locus to its target, which becomes inactivated in the presence of the silencer.

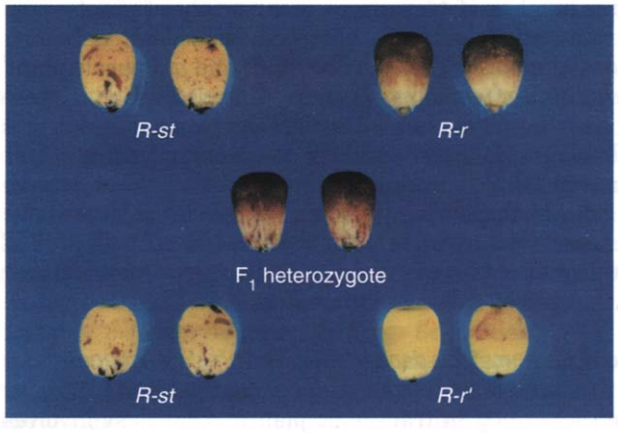

Fig. 3. Maize kernel phenotypes observed as a result of *r* paramutation. A paramutable (target) *R-r* allele can be changed to a weaker, paramutated form, *R-r'*, following an interaction with the paramutagenic (silencing) *R-st* allele, which conditions a spotted phenotype. *Photograph kindly supplied by J. Kermicle.*

response has been found in *Coprinus cinereus*, a basidiomycete fungus that is the most 'evolutionarily advanced' organism yet shown to exhibit true MIP activity[6].

Invasive DNA sequences and cues for de novo *methylation*

Both RIP and MIP demonstrate the central role of methylation in alleviating the potentially harmful effects of invasive DNA. Intrusive sequences could trigger *de novo* methylation during or subsequent to integration if multiple copies of a sequence are present[7]. The DNA methyltransferase enzyme may be able to recognize integration intermediates, such as hairpins, which form when transposable elements or transgenes insert into a genome[7]. Pairing between multiple homologous DNA sequences can also provoke *de novo* methylation. The MIP process in *Ascobolus* appears to involve a pairing signal, because the pattern of methylation is exactly coextensive with a duplicated DNA region and always occurs in both copies (i.e. it never happens that just one copy is methylated[5]).

Plant transposable elements and methylation

Transposable elements are acted upon by the methylation machinery in the plant nucleus. DNA elements [e.g. *Ac* ('activator'), *Spm* ('suppressor-mutator') and *Mu* ('mutator')] undergo reversible inactivation associated with methylation[8], and communication of methylated states can occur between homologous transposable elements in non-allelic (ectopic) positions[9]. Retroelements (DNA sequences that transpose through reverse transcription of an RNA intermediate) are usually highly methylated[10]. An element that is transpositionally active could become methylated when integrating into the genome[7]. The coordinate methylation of unlinked copies of *Mu* elements in maize possibly occurs by means of ectopic pairing, as suggested by methylation being restricted largely to the elements and not flanking plant DNA (Ref. 9). Some infiltration of methylation from transposable elements into flanking plant sequences has also been observed[11,12].

Trans-silencing of transgenes and endogenous genes in plants

Homology-dependent gene silencing has been reported for a number of transgenes in a variety of plant species. Paramutation has been observed for several endogenous genes in maize [*r* ('red'), *b* ('booster') and *pl* ('purple plant')], *Antirrhinum majus* and tomato[8]. Here, attention is focused on two systems that show strikingly similar epigenetic behaviour: promoter homology-dependent silencing of transgenes in tobacco and paramutation at the *r* locus in maize. In both of these cases, a 'silencing' allele or locus induces increased methylation and a heritable reduction in the activity of a 'target' allele or locus; the silencer remains unchanged by the interaction (Fig. 1).

Promoter homology-dependent silencing of transgenes

Two classes of homology-dependent gene silencing have been identified in transgenic plants[1]. One class involves a post-transcriptional process (presumably cytoplasmic RNA turnover) and does not induce substantial methylation of promoter regions or a heritable reduction in gene activity[13]. A second class is associated with transcriptional inactivation, increased promoter methylation and meiotically heritable reductions in gene activity that persist in the absence of the original silencing stimulus[2,14,15]. Because this latter process induces heritable alterations in gene expression, it most closely resembles the paramutation of endogenous genes.

Two promoter homology-dependent silencing loci that are able to *trans*-inactivate and methylate genes at unlinked target loci in tobacco have been identified and analyzed in more detail. One of these loci, *H2* ('hygromycin resistance'), silences genes under the control of the nopaline synthase promoter[16]. The second locus, *271*, is a potent silencer of genes that are under the control of the 35S promoter of cauliflower mosaic virus[15,17]. Both of these loci contain multiple copies of the respective transgene construct (Fig. 2); single copies of the same construct do not have silencing activity[16]. Although the exact arrangement and completeness of the multiple copies are not yet known, silencing activity appears to be associated with methylation in the promoter regions that are the main region of homology to the target locus[16,17]. Spontaneous methylation of the silencing locus possibly occurs when multiple copies of the transgene construct at the locus pair in a process related to MIP. The *cis*-methylated silencing locus would then be capable of imposing methylation on the target locus in *trans* via pairing of homologous promoter regions, a process termed 'epigene conversion'[16]. *Cis*-inactivation is used to refer to the silencing of closely linked genes on the same DNA molecule, while *trans*-inactivation involves homologous genes on different DNA molecules. These genes can be at either allelic or ectopic locations. The methylated, silenced target locus does not revert to full activity or completely lose methylation when a silencing locus is crossed out by breeding, which can result in significant epigenetic variability within the target locus in backcross progeny[15,18].

Paramutation at the maize r *locus*

Paramutation is defined as 'an interaction between alleles that leads to directed, heritable change at the locus with high frequency, and sometimes invariably, within the time span of a generation'[19]. It was first identified and characterized at the maize *r* locus. Members of the *r* gene family, including the displaced homologous *b* and *lc* ('leaf color') loci, regulate anthocyanin expression in various plant and seed parts via production of a transcriptional activator of the myc family of helix–loop–helix proteins[20]. Paramutation at the *r* locus involves a heritable reduction in the activity of a sensitive (paramutable) allele, *R-r*, after it has been associated with an inducing (paramutagenic) allele, *R-st* (*st*: 'stippled'), in the heterozygote (Fig. 3). Recent work on the structure and primary sequences of these two alleles has provided information on the features that might underlie this phenomenon.

To date, all *r* alleles that participate in paramutation have been found to be complex, comprising multiple copies of the *r* transcription unit (Fig. 4). The paramutable *R-r* allele contains multiple complete and incomplete *r* genes in both direct and inverse orientation. A complete *r* gene, *P* ('plant color'), is expressed in plant tissues. The *S1* and *S2* ('seed color') genes form an inverted duplication flanking a truncated and rearranged copy of a *doppia* mobile element. This *doppia* element contains sequences normally found in the *P* promoter and confers seed (aleurone)-specific expression on the *S1* and *S2* genes. The *P* gene lacks a *doppia* element[21].

The paramutagenic *R-st* allele contains four complete copies of the *r* gene in direct orientation[22] (Fig. 3). The *Sc* ('self-colored') gene is capable of conferring strong anthocyanin

expression in the aleurone, but is irregularly expressed because of the presence of an *I-R* (*I*: 'inhibitor of aleurone color') mobile element near its 3′ end. The three additional *r* genes, *Nc1*, *Nc2* and *Nc3* ('near-colorless'), together express a lightly mottled aleurone phenotype. With respect to paramutation, an intriguing observation is that a *doppia* element is present in the promoter of each *Nc* gene; *doppia* thus represents the only common element in the promoter of the *S1* and *S2* genes and *r* gene copies in *R-st* (Ref. 21) (W. Eggleston, unpublished). Reducing the number of *Nc* genes within *R-st* not only leads to increased pigmentation by the remaining copies of *Nc* but also to a reduced ability to induce paramutation[23]. Thus, copy number-dependent silencing at *r* (*cis*-inactivation) and paramutagenicity (*trans*-inactivation) appear related.

The *Sc* promoter region in *R-st* is moderately methylated, and the promoter regions of the three *Nc* genes are highly methylated[22]. As the number of *Nc* genes is reduced, the methylation level of the *Sc* promoter decreases such that little or no methylation is detected in simplex derivatives retaining only the *Sc* promoter (W. Eggleston and J. Kermicle, unpublished). Such simplex alleles have also lost all paramutagenicity[23]. Paramutation of *R-r* results in increased methylation in the 5′-ends of the *S1* and *S2* genes (M. Alleman and J. Kermicle, unpublished; cited in Ref. 20). A paramutated *R-r′* allele (paramutated alleles in maize are designated by addition of a 'prime' symbol after the allele name), which is weakly paramutagenic, gradually recovers activity and becomes less paramutagenic over a few generations in the absence of the *R-st* allele.

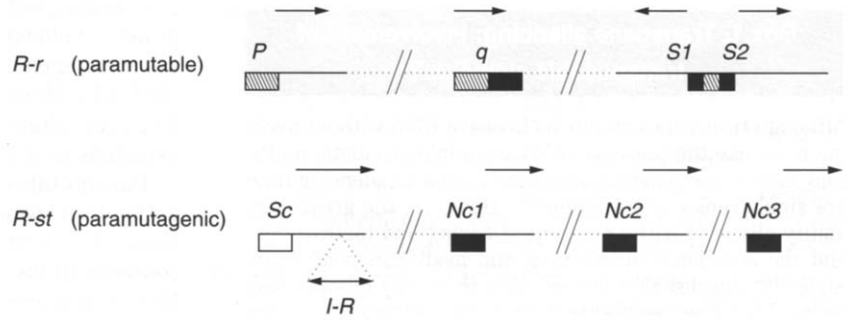

Fig. 4. Structures of paramutable (target) *R-r* and paramutagenic (silencing) *R-st* alleles. Both complex alleles contain multiple copies of the *r* gene. The four tandemly arranged copies at *R-st* (*Sc* and *Nc1–3*) are separated by several kilobases. The *Sc* gene contains an *I-R* transposable element, which plays no role in paramutagenicity[23]. The *R-r* allele comprises three functional *r* genes[21] (*P*, *S1* and *S2*), and *q*, which is homologous to the 5′ region and promoter fragments of *P* (hatched region) but does not contain an *r* coding sequence (solid lines). The distance between *P* and *q* is approximately 190 kb (Ref. 21). The *doppia* transposable elements (filled boxes), which are proposed to mediate the paramutagenic interaction between *R-r* and *R-st*, are present in *q* and in the promoter regions of *S1*, *S2* and *Nc1–3*. The *S1* and *S2* genes form an inverted repeat centred around a rearranged *doppia* element containing sequences homologous to the *P* promoter[21]. The promoter regions of the *Sc* and *Nc1–3* genes at *R-st* are moderately and highly methylated, respectively. The 5′ ends of the *r* genes at *R-r*, including *q*, are increasingly methylated following an association with *R-st*. One-way horizontal arrows indicate the orientation of the genes. The open box is the *Sc* promoter.

Comparisons between promoter homology-dependent silencing of transgenes and r *paramutation*

The similarities between the promoter homology-dependent silencing of unlinked transgene loci and *r* paramutation are inescapable. Both involve:

(1) Silencing and target loci or alleles that share homology in promoter regions; these regions of homology consist of sequences that could be considered invasive DNA (i.e. either multiple copies of transgene promoters or *doppia* elements).

(2) A multicopy silencing locus or allele that autonomously *cis*-inactivates.

(3) A target locus or allele that is weakened in a heritable (but gradually reversible) way during or following an interaction with the silencer.

(4) An association between silencing and methylation.

This similar epigenetic behaviour and involvement of methylation implies a common mechanism based on a methylation/defence system that incapacitates invading DNA.

Recognizing endogenous genes as invasive

It is easy to see how transgenes – even when present as single copies – might be identified as invasive, since they must integrate into a plant genome (Box 1). Nevertheless, multiple copies of a transgene are usually required to incite silencing associated with methylation[2] (although there are exceptions[14,24]), and both loci with strong silencing ability, *H2* and *271*, contain multiple copies of the transgene construct. A high transgene copy number might be considered equivalent to a proliferating sequence by the methylation machinery in the nucleus.

Endogenous genes may also be recognized as being invasive if they resemble a proliferating entity. Two features could be responsible for this in the case of *R-r* and *R-st* alleles. First, both alleles contain multiple copies of the *r* gene. Second, at least some of these *r* genes are associated with *doppia* transposable elements.

r *paramutation, complex alleles and* doppia *elements*

In principle, the coordinate methylation and inactivation of homologous transposable elements could bring under their control any gene that contains a copy of that element. Although it is tempting to consider the *doppia* elements as prime players in *r* paramutation, the role of multiple copies of the *Nc* genes at the *R-st* allele cannot be overlooked, because one copy of *Nc* together with *Sc* is only weakly paramutagenic[23]. Moreover, substituting *Lc*, which lacks a *doppia* element, for *Nc* has little effect on paramutagenic strength[23]. Therefore, an essential feature for generating paramutagenicity of *R-st* appears to be multiple copies of *r* sequences, at least one of which should contain a *doppia* element in its promoter. The *doppia* elements in the *Nc* genes would be required for the *trans*-inactivation of the *R-r* allele because they provide the only region of homology between the *Nc* promoters and the two *r* genes that are most sensitive to paramutation (*S1* and *S2*). Paramutation could occur when the highly methylated state of the *doppia* elements in

Box 1. Transgene silencing: involvement of transposable elements?

Although transgenes qualify as invasive DNA without needing to invoke the presence of transposable elements, multicopy inserts are generally more susceptible to silencing than are single copies of transgenes[1,2]. However, the great variability observed with transgene silencing and methylation, and the occasional inactivation and modification of single copies[14,24], suggest that factors other than copy number may be involved. One possibility is that silenced transgenes are associated with transposable elements[28]. Because of the ubiquity of transposable elements and their remnants (particularly retroelements) in plant genomes[10,31], it is likely that transgenes frequently integrate near to transposable elements. However, data obtained to date on the sequence of DNA flanking stably and unstably expressed inserts of the same transgene construct in tobacco have not revealed a correlation between proximity to known transposable elements and silenced inserts. Nonetheless, much of the flanking plant DNA appears on DNA (Southern) blots to be moderately repetitive (V. Iglesias and A. Matzke, unpublished) and it is possible that it is derived from transposable elements. Another interesting observation requiring further investigation is that stably expressed transgenes are often in the vicinity of nuclear matrix attachment regions (MARs) (Ref. 47) (V. Iglesias, A. Matzke and S. Michalowski, unpublished). In maize, single copy genes can be embedded within extensive regions of moderately to highly repeated sequences that are highly methylated, with MARs present at repeat/single copy junctions[48]. It may be that MARs are able to modulate the influence of repeated DNA on the expression of both endogenous genes and transgenes.

the *R-st* allele is imposed in *trans* on the less-methylated *doppia* element acting as the promoter for *S1* and *S2*. The likely role of *doppia* in mediating strong *trans*-inactivation is illustrated by the relative insensitivity to paramutation of the *P* gene at the *R-r* allele, which lacks a *doppia* element in its promoter.

Paramutation at other maize loci

Other loci in maize that undergo paramutation do not appear to contain complex alleles like those found at the *r* locus. Moreover, there is no obvious correlation either with a specific transposable element or with methylation. In the case of *pl*, another anthocyanin-regulating gene, three different simplex alleles – *Pl-Rh* (*Rh*: 'Rhoades') (a strongly expressed allele), and two variably and weakly expressed derivatives, *Pl-bl* (*bl*: 'blotched') and *Pl'-mah* (*mah*: 'mahogany') – have a *doppia* element in their promoters[21,25,26]. The *Pl'-mah* allele can paramutate *Pl-Rh* (Ref. 26), although this is not associated with increased methylation; however, *Pl-bl* does not participate in paramutation (V. Chandler and J. Hollick, pers. commun.).

Transposable elements are present in the promoter regions of paramutable and paramutagenic *b* alleles but their relationship to paramutation is unclear because a paramutation-insensitive allele, *B-Peru*, contains many of the same sequences (V. Chandler, pers. commun.). The paramutagenic activity of the *B'* allele has been mapped to the 5′-flanking region and transcription of the paramutated gene is reduced 20-fold[27]. Both of these features are reminiscent of promoter homology-dependent silencing of transgenes, which also involves increased promoter methylation[15]. Following extensive testing, however, no methylation changes have yet been correlated with *b* paramutation (Ref. 27). Heritable silencing of both *b* and *pl* may result from an altered chromatin structure, or methylation at cytosines that have not yet been examined[27].

Paramutation at *r* may represent a special type of interaction that involves a paramutagenic allele comprising multiple, closely linked copies of a gene, one or more of which contains in its promoter a particular transposable element that is also present in the paramutable allele. In the case of the *R-st* allele, this configuration might potentiate *cis*-inactivation associated with methylation, and increase the likelihood of pairing with the copies of *doppia* in the *R-r* allele. Consistent with this proposal is the finding that two *r* genes present on each of two chromosomes are less paramutagenic than four *r* genes on a single chromosome[23]. These considerations may also apply to the transgene silencing loci, *H2* and *271*, which likewise have probably acquired methylation and strong silencing ability because of multiple tandemly arranged copies of the transgene construct. In those cases of paramutation that do not involve complex alleles and methylation, it is conceivable that transposable elements are involved in other ways. For example, the elements could generate certain rearrangements of plant DNA, such as inverted repeats, which might in turn promote paramutagenic interactions[28].

Paramutation-like behaviour has been observed for other transposable element-associated genes in maize: the phenotypes of mutations resulting from insertions of defective *Mu* and *Spm* elements can be sensitive to the presence or absence of autonomous members of these two families at other locations in the genome. These effects can also involve coordinate changes in methylation of unlinked transposable elements[12,28,29]. The possible involvement of *Tam* ('transposable element from *Antirrhinum majus*') transposable elements in paramutation of two simplex *niv* ('nivea') alleles encoding chalcone synthase in *Antirrhinum majus* has also been proposed; however, the basis of this interaction is still unclear, as loss of the *Tam* element from one allele does not seem to interfere with paramutation[30,31]. Apparent cases of paramutation involving nonallelic endogenous genes in maize[32] and *Arabidopsis*[33] have also recently been reported, with discussion of the possible role that transposable elements play in these processes[28].

Transposable elements and requirements for differential gene silencing

The silencing of transgenes and some transposable element-associated endogenous genes have been discussed as pathological cases that reveal the action of a genomic 'immune' response to invasive DNA. Because of the ubiquity of transposable elements and transposable element-derived repeats in eukaryotic genomes[8,9], it is worth considering whether some of these elements might play a more general role in regulating plant gene expression.

Gene silencing is an essential part of eukaryotic gene regulation, as up to 50% of the structural genes must be silenced in differentiated cell types[34]. Conceivably, the spread and accumulation of transposable elements and their insertion adjacent to and/or into promoters of structural genes could contribute to the extensive silencing that is required in differentiated cells. Depending on

their distribution, abundance and degree of homology, these sequences could participate in the differential regulation of a 'generic' protein-coding genome by directing differential gene silencing through networks of homologous pairing.

Recent work has uncovered transposable elements (primarily retrotransposons and miniature inverted-repeat transposable elements) or their remnants in the 5′- or 3′-flanking regions of many wild-type plant genes[35]. Most of the retrotransposon sequences appear to have diverged sufficiently such that pairing between multiple copies might be prohibited. Miniature inverted repeat transposable elements (it is not yet known whether these transpose via a DNA or RNA intermediate) are short entities, of about 100–350 bp, that might be unable to pair effectively even if they were highly homologous. Based on these considerations, homology-dependent interactions between these transposable elements might be expected to occur relatively infrequently. However, the length (about 250–300 bp) and homology (>90%) estimates derived from conventional homologous recombination models[36] might not be applicable for DNA homology-based silencing, and alternative possibilities for pairing could also be considered.

Pairing between ectopic DNA elements might be mediated by transposases binding to single ends of two elements at unlinked sites. Genomes of higher eukaryotes might also have a special mechanism requiring a smaller window for recognizing homology than that associated with the standard homologous recombination machinery. The potential for short windows of homology to induce silencing is supported by the observation that only 90 bp of sequence identity in the promoter of the target gene is required for sensitivity to *trans*-inactivation by the *271* silencing locus[17]. Large and complex genomes seem to possess a remarkably efficient homology-searching mechanism, which may even exceed the capabilities found in fungi and yeasts[37]. Finally, repeats too short to promote recombination could provoke silencing if different proteins are involved in pairing and/or if less-intimate pairing is required[38].

Pairing interactions leading to silencing and methylation of homologous sequences could be under developmental control. DNA elements in maize (e.g. *Spm* and *Mu*) can exhibit developmentally regulated methylation and expression[12,28,29]. Genes into which *Spm* elements are inserted can be coupled to the developmental expression pattern of *Spm* (Ref. 29). A group of genes coordinately expressed in maize pollen contains the same retroelement[39]. Other retroelements have been found to be transcribed in specific cell types[10], implying that these sequences are inactive in all other cells, possibly silencing structural genes in their vicinity.

Mammalian imprinted genes recognized as 'foreign'?

Although a phenomenon analogous to paramutation has not yet been observed in mammals, methylation and silencing of DNA recognized as being invasive does occur in these organisms. The progressive silencing of tandem arrays of transgenes under the control of tissue-specific promoters happens frequently in transgenic mice[40]. Retroviruses integrated into promoters of mammalian genes can also cause epigenetic effects not observed with the wild-type gene, such as mosaic patterns of methylation and expression[8,28,41]. Silencing and *de novo* methylation of retroviral vectors used to transduce genes into animal cells have also been reported[7].

Gametically imprinted genes associated with repeats and pairing interactions

Gametically imprinted genes are expressed differently depending on the sex of the parent from which they are inherited. Imprinted genes might be recognized as 'foreign' and thereby be subject to methylation[42]. All known imprinted genes in mammals contain directly repeated sequences, which might form secondary structures comprising the 'foreign' recognition signal[42,43]. The possible role of these repeats has been discussed[43] in relation to a study from *Drosophila* in which a transgene insert adopted a more condensed chromatin conformation as the number of tandemly arranged copies increased, presumably in a pairing-dependent process[44]. A recent study using three-dimensional fluorescent *in situ* hybridization has revealed transient association of imprinted regions on homologous chromosomes[45]. In maize, *r* alleles subject to paramutation also undergo gametic imprinting[19], suggesting a mechanistic link between the two phenomena.

Concluding remarks

A role for transposable elements in paramutation was first suggested by Barbara McClintock[46]. Molecular analyses of *r* alleles involved in paramutation have provided support for this view. However, further work is required to determine whether transposable elements play a role in other cases of paramutation where association with a specific element has yet to be demonstrated. Transgenic plants have offered defined systems with clear parallels to paramutation of endogenous genes and an impetus for examining cases of paramutation involving methylation as a response to invasive DNA.

Two distinct roles for methylation have been proposed[7] to explain the pattern of genomic methylation observed: first, operation as a defence mechanism against foreign or invasive DNA; and second, as a means of controlling gene expression during development. It is becoming apparent that this distinction may not always be clear cut in plants. The extraordinarily similar epigenetic behaviour of some transgene silencing systems and of endogenous genes subject to transposable element-associated paramutation suggests that a defence response to foreign or invasive DNA can also be used against a plant's own genes. A beneficial consequence of the pervasive presence of transposable elements and their remnants in plant genomes might be differential transposon-induced silencing of genes. This implies that the function of methylation as a part of the defence response has merged to a significant degree with its role as a developmental regulator of gene expression in plants.

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