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Genetic elements inducing gene changes at the *pallida* locus of *Antirrhinum majus*

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Summary. The *pal-rec-low-o* is a special repressed state of the *pallida-recurrens* allele, which normally mutates from the recessive to the dominant condition, giving pigmented *Pal* spots on corolla lobes. The *pal-rec-low-o* in the homozygous condition is stably colorless (except for rare mutant spots), but when crossed with the recessive tester strain, *pal-tub pal-tub* (also stably colorless), the mutation frequency of the particular repressed state of *pal-rec* (i.e., *pal-rec-low-o*) increases spectacularly, giving several shifts of varying sizes. The evidence suggests that the activity of the repressed state of the *pal-rec-low-o* allele is dependent on the presence of an independently located *Pr* element, contributed by the *pal-tub* tester strain. In the absence of such a regulatory element, the repressed allele exhibits stable expression due to the effect of a "repressor" *Rp* element residing at or near the locus. It has also been shown that the *pal-tub* regulatory element, *Pr*, while coming through a phase of heterozygosity, could be changed either by picking up an element from the stabilized colorless "original" strain which, being dominant, suppresses the gene action completely; or a change may take place in *pal-tub's* own regulatory machinery, which otherwise has characteristics of gene regulation. However, the *pal-tub* regulatory element, when inactive, can be made trans-active by introducing a fresh regulator into the genome, which may segregate at meiosis.

Key words: Instability – Controlling elements – Mutability shifts – *Antirrhinum majus*

Introduction

In *Antirrhinum majus* several mutable alleles are known for their characteristic unstable phenotypes (Fincham 1970, 1973). Plants homozygous for *pal-rec* (a mutable allele of the *pallida* series) when unstable give *Pal* spots, flakes, or sectors (shifts) of mutability on a recessive background.

Almost a decade ago, an unusual *pal-rec* allele was identified (Sastry 1976) which, when homozygous, appeared to have become more or less completely stabilized in a colorless form. When crossed with the tester strain, *pal-tub pal-tub* (stably colorless), the mutation frequency of the particular *pal-rec* allele increases spectacularly, giving several shifts of varying sizes. This particular state of homozygous *pal-rec* has been designated *pal-rec-low* (Sastry 1976), and its evocation of instability with *pal-tub* was considered to be related to paramutation of *R* in maize, a phenomenon investigated by Brink and his students (Brink 1973).

The present study of the *pal-rec-low* strain (now denoted as *pal-rec-low-o*) showed that *pal-rec-low-o* made heterozygous with *pal-tub* is subject to variation in mutability grades, since the recessive nonmutable *pal-tub* itself is not homogeneous, though the change in the *pal-rec-low-o* gene activity is dependent on the presence of an independently located *Pr* element, contributed by the *pal-tub* tester strain. In the absence of such a regulatory element, *pal-rec-low-o* exhibits stable expression when homozygous, due to the effect of a "repressor" element residing at or near the locus (Aslam 1987). However, in several cases it was found that the *pal-tub* coming through a phase of heterozygosity has changed, either (a) by picking up an element from the so-called stabilized colorless *pal-rec-low-o* (original) strain which, being dominant, suppresses the gene action completely or nearly so; or (b) a change may have taken place in *pal-tub's* own regulatory element, which otherwise has characteristics of gene regulation. Evidence has been presented to show that when the *pal-tub* regulatory element is inactive,

it can be made trans-active by introducing a fresh regulator into the genome, which can segregate at meiosis. An appropriate association of an active *pal-tub* regulatory element with the repressor element of the "original" low line can result in variegation, presumably through switching on and off the actions of the *pal-rec* gene.

Materials and methods

Genetic strains

Antirrhinum strains used were from two different sources. The first group comprises *pal-rec-sd pal-rec-sd* and *pal-tub pal-tub*, and will be referred to as "standard" and tester genetic strains, respectively. Both strains were originally obtained from the John Innes Institute several years ago and have been maintained at Leeds. The main experimental material, *pal-rec-low-o* was established at Leeds from seeds imported from India. Plants derived from the original imported material showed varying degrees of variegation. But two plants turned out to be exceptionally low in mutability, showing one or two tiny spots on corolla lobes in each plant. Seed obtained from those two exceptionally low plants provided a homozygous stock for *pal-rec-low-o* (original), and has been maintained at Leeds in its original homozygous condition by selfing. The *Antirrhinum* stock from both sources, which exhibit different phenotypes corresponding to respective genotypes, are as follows.

Genotype	Phenotype of flower
pal-rec-sd pal-rec-sd (standard)	Flecked with pigment where *pallida* mutates to *Pallida*
pal-tub- pal-tub (tester)	Acyanic except for pigmented ring around the base of corolla tube
pal-rec-low-o pal-rec-low-o (original)	Almost acyanic except for occasional magenta spots on corolla lobes, maintained by selfing
pal-rec-low-nc pal-rec-low-nc (near colorless)	Uniformly low line with occasional magenta spots, derived from the original through crosses
pal-rec-low-o pal-tub	Variegated corolla with a solid magenta ring at the base of the tube
pal-rec-low-nc pal-tub	Variegated corolla with magenta ring at the base of the flower tube
pal-rec-low-act pal-rec-low-act	An established, uniformly high mutable line
pal-rec-low-act pal-tub	A uniformly high mutable line with magenta ring at the base of flower tube

None of the lines carry the known modifier of *pal-rec*, "Stabilizer" (*St*), which reduces the mutability level (Harrison and Fincham 1968).

Quantitative estimates

Mutability level was scored according to a scale consisting of photographs, which showed increasing grades of mutability; on this scale, class 0 represents the flowers with no mutant spots, class 8 means fully colored flowers, and the intervening classes represent the intermediate grades of mutability. Scoring was, however, checked regularly by complete spot counts of representative classes with the aid of a microscope. In general, it was not possible to use the microscopic procedure for scoring several hundred plants within a limited time. But by repeated scoring of coded samples, it was found that our estimates were reproducible within an interval of 0.5 class, so any differences less than that were not considered to be meaningful. In practice, several flowers in an inflorescence are scored individually, and the mean scores for the plants are calculated from these values. This procedure has resulted in mutability classes such as 3.2 or 5.9, instead of round figures.

Results

The *pal-rec-low-o* allele produces different spectra of mutability when made heterozygous with different *pal-tub pal-tub* testers (Aslam and Sastry 1979). It was also reported that *pal-rec-low-o pal-tub* hybrids with very low mutability scores, when backcrossed to plants homozygous for *pal-rec-low-o*, only produce nonactivated *pal-rec-low-o pal-tub* plants. From these observations it appeared that even the tester line is not homogeneous and there is probably something in the original *pal-rec-low-o* that suppresses the instability of the *pal-rec* gene (Aslam 1987).

To clarify the situation, two extremely low heterozygous (45-453-10 and 45-453-6) individuals of the type mentioned above were selfed to extract *pal-tub pal-tub* plants. Homozygous *pal-rec-low-o* was used to see whether these extracted *pal-tub pal-tub* plants could, in fact, activate the *pal-rec-low-o* gene. No mutability was detected except for a few late mutant spots, characteristics of *pal-rec-low-o* (original). This is in contrast to the control crosses in which *pal-tub pal-tub* tester were used, where several plants became activated (Table 1 a). This difference in *pal-rec-low-o* gene activation between the *pal-tub pal-tub* tester and the *pal-tub pal-tub* segregants could be explained on the basis of the follwoing possibilities. (1) The extracted *pal-tub* plants might have inherited a factor that sustains *pal-rec-low-o* in a repressed condition; the factor concerned will be referred to as *Rp* in further discussion. (2) It is possible that the *pal-tub pal-tub* tester plants themselves contain a factor that activates the repressed *pal-rec-low-o*; on this basis, *pal-tub pal-tub* segregants that failed to activate would be lacking in that element. This element would be referred to as *Pr*. Both *Rp* and *Pr* are envisaged as dominant factors. (3) In addition, the *pal-tub pal-tub* segregants were heterozygous for *Pr* and *Rp* elements, since they produce two types of individuals. The above ideas are further supported by the *pal-tub pal-tub* plants derived from a heterozygous plant 45-453-16, which was characterized by mutability class 2.0 shifting. Only two categories of

Table 1 a–c. Average values showing the effect of *pal-tub pal-tub* extracted from **a** heterozygous low mutables (less than 0.5 class), **b** heterozygous low mutables crossed with tester line, **c** heterozygous high mutables, on homozygous *pal-rec-low-o*

		Cross	F_1 mean score ± SD**		Statistical analysis
a	(i)	*pal-tub pal-tub* (Seg) × *pal-rec-low-o pal-rec-low-o*	0.29 ± 0.00 (193)		$t = -46.27$;
	(ii)	*pal-tub pal-tub* × *pal-rec-low-o pal-rec-low-o**	3.05 ± 0.00 (40)		$df = 9$; $P < 0.001$
			Score of high mutables	Score of low mutables	
b	(i)	*pal-tub pal-tub* (Seg) × *pal-rec-low-o pal-rec-low-o*	3.55 ± 0.36 (64)	0.31 ± 0.13 (53)	χ^2 (for low and high) = 1.03;
c	(i)	*pal-tub pal-tub* (Seg) × *pal-rec-low-o pal-rec-low-o*	4.83 ± 0.24 (192)		$t = 6.26$; $P > 0.05$
	(ii)	*pal-tub pal-tub* × *pal-rec-low-o pal-rec-low-o**	2.95 ± 0.35 (80)		$df = 10$; $P < 0.01$

* Control cross
** Number shown in brackets indicates number of plants scored

pal-tub pal-tub segregants were tested when it was selfed. One category of segregants (such as 45-627-4) produced about 50% of reasonably high mutables (class 2.80 ± 0.83) when crossed to homozygous *pal-rec-low-o*, while the rest of them were found to be colorless. Closer examination of the latter revealed a few late mutant spots. The second category of *pal-tub pal-tub* (segregants of 45-627-1) extracted from the same mutable individual produced plants of only near colorless phenotypes (Fig. 1).

From these findings, it appears that the first category of *pal-tub pal-tub* segregants was carrying two independent factors (*Pr* and *Rp*) responsible for *pal-rec-low-o* gene activation and repression, respectively: hence, when segregated at meiosis these segregants produced two types of progeny. But the second categroy of *pal-tub pal-tub* segregants was either homozygous for the "repressor" (*Rp*) factor or lacked both a repressor and an active *Pr* regulatory element and, hence, these segregants were inert. This was further substantiated by the fact that when *pal-tub pal-tub* segregants from a low heterozygous plant (45-453-10, of mutability class 1.0) were crossed to homozygous *pal-rec-low-o*, only nonactivated (i.e., near colorless) progeny were produced (Table 1 a).

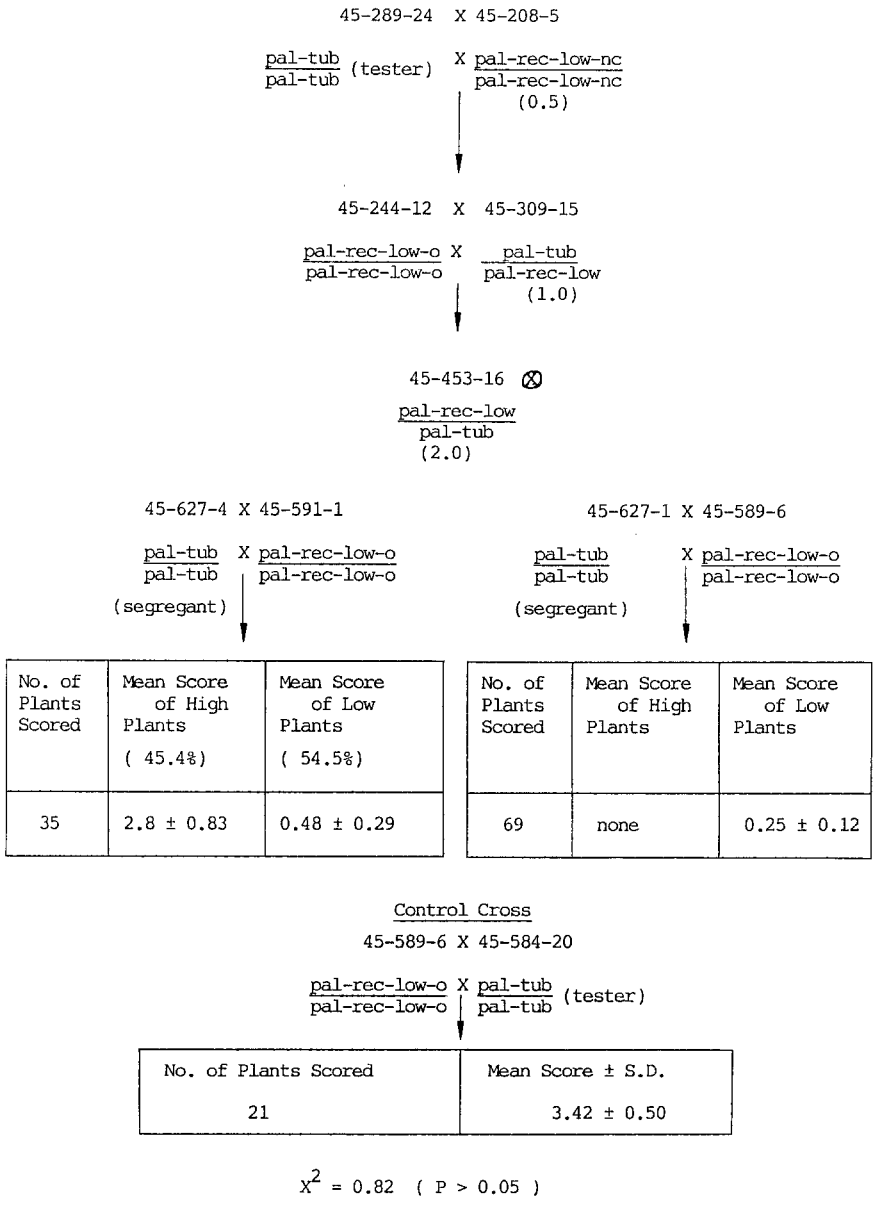
Part b of Table 1 supports the hypothesis that non-*pal* factors are involved. Assuming that the heterozygous low mutable used carries an *Rp* (repressor) then, when such a mutable was crossed with the *pal-tub pal-tub* tester, some segregants (*pal-tub pal-tub*) would obtain *Rp* from the low mutable and an active *Pr* regulatory element from the *pal-tub* tester stock, to produce bifactorial segregants. Such segregants produce two types of progeny when crossed to homozygous *pal-rec-low-o*, indicating independent segregation of two at meiosis. Alternatively, if it is assumed that the particular low mutable (45-453-10) lacked an active *Pr* regulatory element in its genome, with the introduction of a fresh element through the *pal-tub pal-tub* tester the mutant was made trans-active, segregating out at meiosis and thus producing high and extremely low individuals.

Interestingly enough, *pal-tub pal-tub* plants extracted from very high activated *pal-rec-low-act* lines showed a higher activation of the *pal-rec-low-o* gene than that produced by *pal-tub pal-tub* tester. When crossing *pal-tub pal-tub* segregants, the low original line produced progeny with a 4.83 ± 0.24 score; *pal-tub pal-tub* tester produced only class 2.95 ± 0.35. The difference of almost two classes is highly significant (Table 1 c).

Specificity of Rp and Pr elements

It is possible that *Rp* and *Pr* might affect only *pal-rec-low-o* allele and not *pal-rec-sd*. To test this possibility, *pal-rec-low pal-tub* plants with extremely low mutability (less than class 0.5) were crossed to (a) *pal-rec-sd pal-rec-sd*, and (b) *pal-rec-low-act pal-rec-low-act* lines. Line (a) is fairly homogeneous, with an average score of 6.5; no low plants were produced by this line by the time the crosses were made. *pal-tub pal-tub* tester × *pal-rec-low-act pal-rec-low-act* and *pal-tub pal-tub* tester × *pal-rec-sd pal-rec-sd* crosses were used as controls.

As suspected, *pal-rec-sd* is not sensitive to the action of either the *pal-rec-low-o* allele or to that of the segregating *pal-tub*. This is clearly shown by the fact that the scores registered by *pal-rec-low pal-rec-sd*, *pal-rec-sd pal-tub* (seg), and *pal-rec-sd pal-tub* (tester) are not significantly different (Table 2 a). In contrast to this, *pal-tub* segregants have positively reduced the mutability of the *pal-rec-low-act* allele, as shown by the reduction of about 3.0 classes as compared to the control crosses (Table 2 b). As can be seen from Table 2 b, *pal-rec-low pal-rec-low-act* genotypes also registered lower scores than those produced from control crosses, but it is difficult to say how much reduction is contributed by the *pal-rec-low* allele per se and how much by other factors (such as *Rp*). However, when highly activated *pal-rec-low-act pal-tub*



No. of Plants Scored	Mean Score of High Plants (45.4%)	Mean Score of Low Plants (54.5%)
35	2.8 ± 0.83	0.48 ± 0.29

No. of Plants Scored	Mean Score of High Plants	Mean Score of Low Plants
69	none	0.25 ± 0.12

No. of Plants Scored	Mean Score ± S.D.
21	3.42 ± 0.50

Fig. 1. Derivation of *pal-tub pal-tub* plants extracted from a mutable, 45-453-16, and their crosses with homozygous *pal-rec-low-o* to show that the mutation-inducing factor (*Pr*) is segregating, since the low mutable carries only one *Pr*

Table 2a and b. Average scores registered by the progeny produced by **a** *pal-rec-low pal-tub* × *pal-rec-sd pal-rec-sd*, **b** *pal-rec-low pal-tub* × *pal-rec-low-act pal-rec-low-act*, and their respective control crosses

	Cross	Progeny score ± SD **		Statistical analysis
		pal-tub (seg) pal-rec-sd	*pal-rec-low pal-rec-sd*	
a (i)	*pal-rec-low pal-tub* × *pal-rec-sd pal-rec-sd*	5.07 ± 0.40 (184)	5.50 ± 0.45 (202)	$t = -3.26$; $df = 21$; $P > 0.001$
(ii)	*pal-rec-sd pal-rec-sd* × *pal-tub pal-tub* *	5.75 ± 0.15 (80)		
		pal-tub (seg) pal-rec-low-act	*pal-rec-low pal-rec-low-act*	
b (i)	*pal-rec-low pal-tub* × *pal-rec-low-act pal-rec-low-act*	2.48 ± 0.27 (132)	3.90 ± 0.45 (126)	$t = -14.42$; $df = 9$; $P < 0.001$
(ii)	*pal-rec-low-act pal-rec-low-act* × *pal-tub pal-tub* *	5.43 ± 0.12 (40)		

* Control cross

** Number shown in brackets indicates number of plants scored

(class 5.0) were crossed to homozygous *pal-rec-sd*, neither segregant *pal-tub* nor the *pal-rec-low-act* affected the standard (*pal-rec-sd*) allele. What is more interesting, *pal-rec sd pal-rec-low-act* heterozygotes produced a variegated pattern that is significantly different ($p > 0.05$) from that of *pal-rec-sd* homozygotes, suggesting that *pal-rec-low-act* is free from all mutation-affecting factors and behaves like the *pal-rec-sd* allele.

Discussion

This project was undertaken to investigate the genetic basis of a repressed phenotype shown by plants of *pal-rec-low-o* genetic background. Some time ago, it was reported that this particular repressed state of *pal-rec* (*pal-rec-low-o*) showed various grades of mutability (in terms of shifting) when made heterozygous, particularly with the *pal-tub* tester strain (Sastry 1976). Within F_1 individuals, reversion towards the original state was also found, and this phenomenon was related to paramutation of *R* in maize, a system originally described by Brink (1973).

The experimental results in the present report indicate that the *pal-rec-low-o* made heterozygous with the *pal-tub* (tester) is subject to de-repression resulting in various mutability grades, but the changes appear to be rather erratic. A search for a genetic basis resulted in finding two categories of elements – the regulator *Pr*, contributed by the stably recessive *pal-tub* and a repressor *Rp*, contributed by the "low original." Their characteristics and specific relationship with one another have been the subject of the present investigation.

It was already demonstrated elsewhere (Aslam 1987), that the *pal-rec-low-o* gene function is determined by the activity of the *Rp* element, which is resident to the "original" line. On making plants of "near colorless" phenotype heterozygous with the *pal-tub* tester, the factor originally responsible for repression presumably moves away from the locus of the gene and can be segregated with *pal-tub*. An appropriate association of such a stabilizing factor with another factor, *Pr*, of a destabilizing nature, contributed by *pal-tub* through phase of heterozygosity, may result in plants with sectorial appearance (shifting). To substantiate this hypothesis, *pal-rec-low-nc pal-tub* hybrids were crossed back to *pal-rec-low-o*; the resulting heterozygotes only produced plants with low phenotype, indicating loss of *pal-tub* capacity to induce a mutational change in activity of the *pal-rec-low-o* gene. In the light of these observations, the activity of a *pal-tub* in association with *pal-rec-low-o* can be criticized. Initiated with *pal-rec-low pal-tub* plants with very low phenotype (less than 0.5), no evocation is either due to (a) loss of sensitivity of *pal-rec-low* to undergo changes, or (b) *pal-tub* losing its "ability" to induce the change. Crossing with fresh *pal-tub pal-tub* tester showed that *pal-rec-low* can still undergo the change. The *pal-tub pal-tub*, on the other hand, has in many cases failed to induce the change in homozygous *pal-rec-low-o* plants; the same *pal-tub pal-tub* plants have reduced the mutability of *pal-rec-low-act* but not the *pal-rec-sd*.

On the basis of such a specific genetic control of mutability, observations on *Antirrhinum majus* with special reference to *Pr-Rp* elements can be compared with maize systems. For example, in the *Ac-Ds* system of gene control in maize (McClintock 1956), it was found that in the absence of *Ac* (a regulatory element), no changes affecting gene action occurred, and stability of gene expression was exhibited as long as *Ac* was not present. In several other cases described by McClintock (1962) and Peterson (1970), a mutable locus originally closely associated with the controlling element loses its capacity to mutate autonomously; the locus itself becomes stable, showing either no mutability or very low levels of gene expression. This may still, however, mutate in response to the presence of a controlling element elsewhere in the genome. This observation led McClintock (1956) to propose that controlling elements in their complete form comprise two separable components, one component, the "receptor", receiving and responding to signals coming from the second component, the regulator. From this, McClintock (1962) concluded that the initially autonomously mutable locus has become dependent for its mutability on an external regulator element, only because of the transposition of the regulator to elsewhere in the genome, leaving the receptor alone at the mutable locus. An opposite situation has also been reported (McClintock 1965), when loss of mutational response presumably due to loss of the receptor, with retention of the regulator, has been shown by the presence of an element at the original locus capable of inducing mutations at other loci susceptible to its action.

Knowing that in *Antirrhinum majus, pal-rec-low-o* is activated when crossed with a *pal-tub pal-tub*, the question is: what makes the *pal-rec-low-o* responses vary so markedly with different *pal-tub pal-tub* testers? This can be attributed to several causes. (1) The presence of a single *Pr* regulatory element in the *pal-tub* genome could be the reason for high and low plants in F_1 progeny, as is true of the *Ac* regulatory element in maize (McClintock 1956). (2) *pal-tub* regulatory functions in some cases could be of a heterozygous nature (active- and inactive-inducing effect), corresponding to cyclic changes in the activity of the element (Aslam and Sastry 1979). In other words, hybrids showing very low or no levels of gene expression have received a changed *Pr*. Such an inefficiency of the *pal-tub* regulatory element, *Pr*, was observed in several cases (Fig. 1). This suggests that simple heterozygosity, as claimed by Sastry (1976), does not

cause a change in *pal-rec-low-o* gene activity, but rather that control of mutability (regulation and suppression) is due to the mutual activity of the *Pr* regulator and its responsive *Rp* element. However, it has also been demonstrated (Table 1 b) that the activity of an initially inactive *Pr* regulatory element can temporarily be restored if an active regulator is introduced, which may segregate at meiosis. These observations reflect some similarities with bacterial systems of gene control, with special reference to *Insertion Sequences* (*IS* elements) when *IS2*, like that of the *Pr* regulator, reduces operon expression when integrated in a particular orientation; and when it is in opposite orientation, genes located down stream are expressed constitutively at a level three times higher than normal (Nevers and Saedler 1977; Saedler et al. 1974), whereas *IS1* behaves like the *Rp* (repressor) element and reduces operon expression when integrated in either orientation.

In maize also, it has been reported that an inactive element can be temporarily activated when a second, fully functional element is introduced into the genome. The originally inactive element was not turned on permanently in this process, however, and ceased to be expressed when its active counterpart was removed by meiotic segregation (McClintock 1971). The inactive *Pr* regulator, like the *Ac* element in maize, although unable to induce changes of the *pal-rec-low-o* gene presumably through excision event of the responsive element, *Rp*, has not lost its own ability to move, although no test of the hypothesis was carried out to show that these elements are movable. However, the fact that such hypothetical movements of the inactive *Pr* regulator could occur in response to the active *Pr* in the nuclei of a plant does not necessarily result in restoration of activity of the inactive *Pr* regulatory element. It segregates from the active *Pr* at meiosis and may be recovered in the progeny.

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