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SPECIFIC CONTROL OF MUTABILITY BY THE *SFM* ELEMENT OF GENE REGULATION IN *ANTIRRHINUM MAJUS*

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Abstract

The *Sfm*, a regulatory element controls the mutability of standard *pal-rec-sd* allele in the form of shifts in *Antirrhimum majus*. Another allele of the *pallida* series shows an extremely reduced, or no level of instability when homozygous; an heterozygous condition of which with *pal-tub pal-tub* tester can bring about changes of the "low" gene in the form of mutability shifts. Evidence have been presented to show that the introduction of *Sfm* element into the genome of plants of *pal-rec-low-o* genetic background has completely failed to induce shifting or even to evoke the instability of instability; whereas it allows full gene expression if combined with the activated *(pal-rec-low-act)* allele of the same strain. This points out the specificity of the *Sfm* element for a gene of particular status.

Introduction

The controlling elements are known to integrate at a number of sites in the chromosome (Daniel *et al.*, 1972; Finnegan *et al.*, 1978). When inserted within a gene, they greatly reduce or completely eliminate transcription of the gene (Daniel & Abelson, 1973; Starlinger & Saedler, 1972). Excision of such elements results in restoration of gene activity, but this depends on the mode of excision event, i.e., if deletions of these are left behind they would account for mutation either to full gene activity or to one of a series of quantitatively graded alleles (Fincham & Sastry, 1974). An altered state of the *Sfm* or probably its improper insertion into the gene locus may bring about changes of state, leading to phenotypic modifications.

The *Sfm* element shows no changes of the *pal-rec-low-o* gene if inserted into it; whereas it allows full gene activity if associated with the activated *(pal-rec-low-act)* allele of the same strain. This points out the specificity of the element, which presumably depends on the specificity of the responsive element. In the absence of a specific responsive element, the regulatory functions may not necessarily be transcribed. The inactive *Sfm* is inactive only with regard to dose effect and instability -inducing capacity, both of which appear to reflect one component of action of the *Sfm*. It continues to produce a particular pattern of shifting in plants of *pal-rec-low-act* or *pal-rec-sd* genetic background. The specific control of mutability by the *Sfm* element of gene regulation in *Antirrhinum majus* is presented.

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Table 1. Phenotype exhibited in three generations produced by selfing a *pal-rec-JI pal-tub* (1452-7 plant with shifting).

Gene-ration	Parental pedigree and level of shifting	Progeny	Heterozygous plants showing Shifting %*	Non-shifting%*	Average score	Homozygous plants showing Shifting %*	Non-shifting %*	Average score
1.	1452-7 (class 6.5)	V32	53.33(8)	46.66(7)	5.7±1.03	50.00(1)	50.00(1)	6.25±0.64
2.	V32-2(class 5.5)	45-342	(100.00(15)	00.00(0)	4.46±1.54	60.00(3)	40.00(2)	5.50±0.70
	V32-12(class 6.0)	45-343	100.00(11)	00.00(0)	4.86±0.71	60.00(3)	40.00(2)	6.10±0.65
3.	45-342-3(class 4.0)	45-522	85.71(6)	14.28(1)	5.85±0.69	50.00(1)	50.00(1)	6.50±0.18
	45-342-5(class 4.5)	45-526	77.77(7)	22.22(2)	6.38±0.33	33.33(1)	66.66(2)	7.00±0.00
	45-343-17(class 6.1)#	45-533				20.00(3)	80.00(12)	6.03±0.18
Mean			83.36(47)	27.72(10)	5.45±0.77	45.55(12)	54.55(20)	6.38±0.56

*: Number of plants scored in each case are given in brackets.
#: Plant used was homozygous for pal-rec-JI.

Statistical analysis:

χ^2 values for pal-rec-JI pal-tub = 1.69 $P > 0.05$
χ^2 values for pal-rec-JI pal-rec-JI = 2.67 $P > 0.05$
χ^2 values for pal-rec-JI pal-tub and pal-rec-JI pal-rec-JI = 3.60 $P > 0.05$

Materials and Methods

The genetic strains of *A. majus* were obtained from two different sources. The first group comprizes *pal-rec-sd pal-rec-sd* and *pal-tub pal-tub* and will be referred to as "standard" and "tester" genetic strains respectively. A derivative of *pal-rec-sd* which showed regular shifts of mutability (sectors of mutability on a recessive colourless background) will be referred to as *pal-rec-JI pal-rec-JI*. The second group comprizes *pal-rec-low-o pal-rec-low-o* and *pal-rec-low-act pal-rec-low-act;* the description of their characteristic phenotypes is given elsewhere (Aslam, 1987).

The material for these lines was imported from India. Plants derived from the original imported material showed varying degrees of variegation. But exceptionally low mutables (almost colourless except for one or two tiny spots occasionally found on corolla lobes) provided a homozygous stock for *pal-rec-low-o* (original) and has been maintained at Leeds in its original homozygous condition by selfing. The *pal-rec-low-act pal-rec-low-act* is a uniformly established high line obtained by crossing the original with *pal-tub pal-tub* tester and selecting. Floral instability estimates for anthocyanin pigmentation were obtained by scoring individual flowers against a standard scale consisting of 0-8 classes. Flowers with no mutant spots were considered as to represent class 0 whereas, fully coloured phenotypes represented class 8 and the intervening classes represented the intermediate grades of instability.

Results

When a *pal-rec-sd pal-tub* hybrid plant with clear shifting, 1452-7 was selfed, the progeny particularly heterozygous, showed a high frequency of plants with shifting (Table 1). None of the plants were uniformly low, as can be seen from high scores in Table 1, and the segregation pattern of shifting is Mendilian, suggesting the presence of a dominant shifting factor *Sfm* (Aslam, 1990). This behaviour was repeated through out three selfed generations observed in the present study. Following the isolation of *Sfm* through crosses of *pal-tub* with John Innes shifting line, it was interesting to see if it has any effect on mutability of the *pal-rec-low-act*. As has been shown (Aslam, 1987) *pal-rec-low-o* exhibits extensive shifting in early generations after activation with *pal-tub pal-tub* tester. By repeated selfing and selecting, several *pal-rec-low-act* lines were produced with little or no shifting. The question is whether the introduction of *Sfm* in such lines results in shifting? The experiments were carried out with two sets of crosses: in the first case, homozygous *pal-rec-low-act* with high scores were crossed with *pal-rec-sd pal-tub* containing one *Sfm*. The data presented in Table 2 shows that several plants in the F_1 progeny showed shifting (Fig. 1) but the process of shifting itself did not show any clear cut Mendelian segregation. This segregation pattern has been further clouded by the fact that 10% of the plants in control crosses exhibited clear shifting. This indicated *pal-rec-low-act* obviously has not completely lost the shifting ability when made heterozygous with *pal-tub*.

Table 2. Effect of Sfm on the mutability of Established pal-rec-low-act: Homozygous pal-rec-low-act plants were crossed with pal-rec-sd pal-tub containing one Sfm.

Cross No.	Cross	Genotype	pal-rec-low-act / pal-tub score	% of pal-rec-low-act / pal-tub shifting*	pal-rec-low-act / pal-rec-sd score	% of pal-rec-low-act / pal-rec-sd shifting*	Mean score + S.D.
1.	45-1072-14 x 45-1156-9	pal-rec-low-act x pal-rec-sd pal-rec-low-act pal tub	4.20±0.75	80.00 (5)	5.10±0.54	60.00 (10)	4.78±0.75
2.	45-1155-6 x 45-1219-5	pal-rec-sd x pal-rec-low-act pal-tub pal-rec-low-act	4.73±0.56	82.00 (29)	5.76±0.50	11.76 (17)	5.12±0.73
3.	45-1155-6 x 45-1219-10	pal-rec-sd x pal-rec-low-act pal-tub pal-rec-low-act	4.96±0.60	96.00 (29)	5.83±0.44	37.50 (16)	5.26±0.69
4.	45-1218-13 x 45-1178-2	pal-rec-low-act x pal-tub pal-rec-low-act pal-rec-sd	5.10±0.22	66.60(9)	5.77±0.46	18.00 (11)	5.47±0.49
5.	45-1218-10 x 45-1152-14	pal-rec-low-act x pal-tub pal-rec-low-act pal-rec-sd	4.96±0.94	83.00 (12)	5.54±0.41	63.00 (11)	5.19±0.79
6.	45-1154-4 x 45-1219-2	pal-tub x pal-rec-low-act pal-rec-sd pal-rec-low-act	5.31±0.37	75.00 (8)	6.31±0.25	25.00 (8)	5.81±0.60
Mean			4.87±0.34	80.00 (92)	5.71±0.39	35.80 (73)	5.27±0.34
7.#	45-1219-2 x 45-1201-1	pal-rec-low-act x pal-tub tester pal-rec-low-act pal-tub	5.70±0.34	10.00 (20)	—	—	5.70±0.34

*: Number in brackets indicates number of plants scored in each case. #: Control cross.

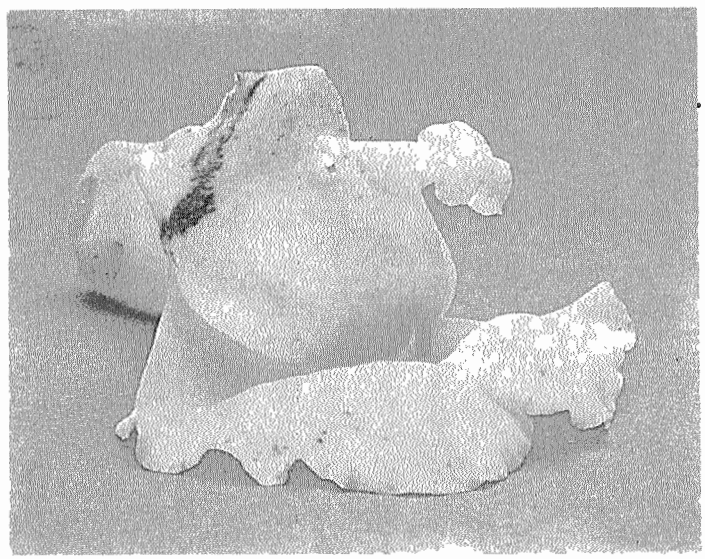

Fig. 1. Shifting as shown by *pal-rec-low-act pal-tub Sfm* (one) genotype.

Another interesting feature of these crosses is that about 9% of F_1 plants were found to be colourless excepting for an occasional mutant spot. Although, their nature has not been completely investigated further, it is tempting to postulate that the plants in question might have received a changed *Sfm* (non-transposing?). Similar colourless plants were also found in the progeny from *pal-tub Sfm pal-rec-sd*. Side shoots of several colourless plants have been examined to investigate the plant variation. None of the branches showed any difference from their respective main shoots.

In the second set of crosses, homozygous *pal-rec-low-o* were crossed with *pal-rec-sd pal-tub* with one dose of *Sfm*. The progeny did not produce plants with mutable phenotypes showing that *pal-rec-low-o* was not activated. However, occasional individuals with small mutant sectors have been observed. This could simply mean those *pal-rec-low-o* plants which have not received *pal-tub* or *pal-rec-sd* with *Sfm* showed mutability.

Discussion

The question of considerable interest concerns the specificity of the *Sfm* controlling element. Firstly, it has been shown to be effective only for *pal-rec-sd* or for *pal-rec-JI* gene of John Innes line, from which it was originally extracted. But later, its effects as a general mutator were also observed on activated *pal-rec-low-act* lines which produced shifting. But the amount and frequency of shifts induced by the *Sfm* in progeny of *pal-rec-low-act pal-rec-low-act* X *pal-rec-sd pal-tub (Sfm)*, is far less than that produced with *pal-rec-sd*. These differences in the amount of shifts could be attributed to differences in

the local genomic environment among different strains in which these elements are inserted. This idea of controlling elements gains further support from the investigation of mutations in *Drosophila* which result from the local integration of dispersed repeated gene family element (Potter *et al.*, 1979). A test of the hypothesis carried out by Strobel *et al.*, (1979) showed that the number and chromosomal locations of elements of the "412", "Copia" and "297" dispersed repeated gene families, differed extensively when the genomes of *Drosophila melanogaster* strains were compared. Moreover, it is interesting to note that differences in the arrangement of these elements occured among individuals tested from the same stock. Also, dispersed repeated DNA sequences with elements of similar structure to those of *Drosophila* have been found in the genome of the yeast *Saccharomyces cerevisiae* (Cameron *et al.*, 1979). The yeast elements also appear to be capable of transposition and exhibit different genomic organizations in different yeast strains.

On the other hand, a closely parallel situation exists in bacteria where *IS* elements and *Mu* phage of *E. coli* are known to integrate at a number of sites in the chromosome. When inserted within a gene, they greatly reduce or completely eliminate transcription of the gene (Starlinger *et al.*, 1973). Excision or *Mu* phage of *IS* elements from the *E. coli* chromosome, can result in restoration of gene activity but this depends on the mode of excision event, i.e., if deletions of these are left behind they would account for mutation either to full gene activity or to one of a series of quantitatively graded alleles (Fincham & Sastry, 1974). However, the integration and excision of an element without damage to the gene is not impossible, but such precision is not always the rule and in some cases integration and excision is accompanied by deletions (Reif & Saedler, 1977). Infact, the conclusion was that possibly the sole function of these elements is to promote genetic variability, and that their gene products might only be necessary for the maintenance and mobility of the elements themselves, rather than other cellular processes.

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