Genetic evidence for repression of somatic P element movements in *Drosophila melanogaster* consistent with a role for the KP element

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The P family of transposable elements in *Drosophila melanogaster* has, since its introduction into *D. melanogaster* populations in this century, diversified into a number of internally deleted forms. One of these, the KP element, is abundant in the genomes of flies from populations from Asia, Europe and Africa. There is some evidence that this sequence can act as a repressor of transposition. We have studied a mutation, $singed^{very\,weak}(sn^{vw})$, in which a KP element is one of two P elements inserted into the 5' exon of the X-linked *singed* gene. These elements can be mobilized by a *trans*-acting dominant mutation, *Mo*, with a maternal effect, on the second chromosome. The rate of somatic reversion of sn^{vw} induced by *Mo* is reduced threefold if the mother herself possesses sn^{vw} on her X chromosomes. This implies that sn^{vw} may be responsible for a form of repression of P element movements. The cause of this effect may be related to transcription of the KP element in sn^{vw}. However, an effect of other genomic P elements in the repression of somatic reversion of sn^{vw} cannot be entirely excluded.

Keywords: *Drosophila melanogaster*, P elements, transposable elements.

Introduction

The P family of transposable elements is a class of transposable elements in *Drosophila melanogaster* that move via a DNA mechanism (Engels *et al.*, 1990; Gloor *et al.*, 1991; Nassif *et al.*, 1994). This movement induces a syndrome of traits including male recombination and high temperature sterility that have been called P-M hybrid dysgenesis. Since its invasion of the *Drosophila melanogaster* genome early in this century (Anxolabéhère *et al.*, 1988) the P element has evolved into a heterogeneous family of sequences, most of which differ from the ancestral full-length sequence by internal deletions (for review see Engels, 1989). The full-length element encodes the P transposase, which is required in *trans* for all P element movements. The collections of P elements in the genomes of *D. melanogaster* populations from Europe, Asia and Africa, are dominated by a particular deletion derivative called the KP element (Black *et al.*, 1987). This has a single internal deletion removing nucleotides 807 to 2561 of the 2907 base pair P element sequence (O'Hare & Rubin 1983).

Whereas full-length P elements produce transposase in the germline, a number of systems in which transposition is repressed are also produced by various kinds of P elements. In somatic cells splicing of the transcript from the element is incomplete (Laski *et al.*, 1986; Laski & Rubin, 1989; Siebel *et al.*, 1992), and the third intron is retained. The truncated protein produced, which lacks amino acids specified by the fourth exon of the transposase gene, has also been shown to be a repressor of transposition.

A number of repression systems are known that operate in the germline. These can be monitored by screening the attenuation of the high temperature sterility caused by hybrid dysgenesis, or by looking at the reversion rate of a double P element insertion mutation in the *singed* gene called $singed^{weak}$ (Engels, 1979; Roiha *et al.*, 1988). One germline repression system is P-cytotype, which is maternally inherited. P-cytotype appears often to be the result of a class of P elements which lack some or all of the fourth exon. The structure and properties of these, so-called class I, repressors have received considerable study (Gloor *et al.*, 1993). Sometimes, full-

length P elements appear capable of eliciting P-cytotype by virtue of their genomic position. A naturally-occurring pair of such elements on the tip of the X chromosome have been shown to be capable of causing P-cytotype (Ronsseray *et al.*, 1991). Molecular studies using the P element promoter attached to a *lacZ* reporter gene have shown that P-cytotype results in a reduction in the level of transcription from a P promoter (Lemaitre & Coen, 1991; Lemaitre *et al.*, 1993). There is also evidence that shorter deleted elements can also cause repression of transposition. For these, the evidence argues against a maternally-inherited repression, but for repression that is zygotic or through a maternal effect arising in a single generation. Much the most abundant internally deleted P element in populations from the old world is one called the KP element (Black *et al.*, 1987). The KP element has been found to increase its genomic copy number rapidly in laboratory strains, provided that full-length P elements are present (Jackson *et al.*, 1988). These authors interpret this increase as driven by the natural selection resulting from a repressing KP element's capacity to reduce the sterility effect of hybrid dysgenesis. Simulations have shown that inactive or repressingelements can invade populations of intact dysgenesis-inducing elements under a wide range of conditions (Brookfield, 1991). The data concerning KP elements' capacity to repress P element movements are, however, highly heterogeneous. Some strong P strains, showing no chromosomally-inherited repression, contain large numbers of KP elements (Monastirioti *et al.*, 1988). Other strains, with high levels of chromosomally-determined repression, lack KP elements (Heath & Simmons, 1991). A survey of the repressing properties of single deleted P elements (Rasmusson *et al.*, 1993) showed that although two of the three KP elements tested repressed *singed^{weak}* reversion in the presence of a P transposase source, and a similar length P sequence did not, the strongest repression was elicited by a much rarer and shorter element, called SP. Furthermore, one of three KP elements tested showed no repression. One explanation for the capricious nature of repression could be that it depends upon the level of transcription of the element, which will itself by largely dependent on the presence of transcriptional enhancers near the target site (a phenomenon exploited in the 'enhancer trap' technique (O'Kane & Gehring, 1987)). Similarly, a KP element inserted at 47D on the second chromosome is associated with a strong repression of P element movements (Higuet *et al.*, 1992), but several lines of evidence suggest that this

may be the result of a mutation in a host gene.

In the mutation *singed$^{very\ weak}$* (or *sn^{vw}*), two deleted P elements are inserted into the *singed* gene in a tail-to-tail orientation. The phenotype is very weak curvature of the bristles, less profound than for *singed^{weak}*. This mutation can show what is, for P element insertion mutations, the very unusual property of being somatically unstable. *sn^{vw}* can revert somatically to patches of strong *singed* tissue in a very weakly *singed* phenotypic background, but only in the sons of females which have a dominant maternal effect mutation, which we have called *Mo* (for mosaicism) (Brookfield & Lewis, 1989). The two P elements in *sn^{vw}* are apparently inserted next to each other save for the 8 base pair target sequence duplicated between them, at base pair 1580 of the *singed* sequence (Paterson & O'Hare, 1991; Ortori *et al.*, 1994). This is part of the 5' untranslated exon of *singed* (Roiha *et al.*, 1988; Paterson & O'Hare, 1991). The larger of the two elements is transcribed in the same direction as the *singed* gene, and has an internal deletion from 807 to 2561, which shows it to be a KP element. The smaller, downstream, element is transcribed in the opposite direction to the first and to *singed*, and has an internal deletion removing base pairs 204–2500.

Under the influence of zygotic or maternal *Mo*, or both, the *sn^{vw}* mutation reverts in the male germ line at 2–7 per cent per generation, to give products in which the smaller or the larger element is precisely deleted. These products have strong *singed* (below called *singed^{extreme}*, or *sn^{e}*), and pseudo-wild-type (*sn^{+}*) phenotypes, respectively. The generation of mosaic *sn^{e}* patches is also caused by somatic excision of the smaller element (Ortori *et al.*, 1994).

Mo maps between map positions 59–64 in the second chromosome, which implies that it is in chromosomal division 47. A strong band of P element homology at 47C has been identified through *in situ* hybridization to *Mo* chromosomes (J. F. Y. Brookfield *et al.*, unpublished data). Thus, an economical explanation of the nature of *Mo* is that it is a modified P element which puts a form of P transposase with an usually long half-life into the egg, which then causes precise excisions of the elements from *sn^{vw}* in the zygote.

The combination of *sn^{vw}* and *Mo* suggests a test of the repressor capacity of the KP element. *sn^{vw}* is a highly unstable mutation which includes a KP element. This shows that the presence of a KP element in the cell cannot, by itself, strongly repress all P element movements. However, the repressive effects of the KP element may be dependent on whether it is transcirbed. The advantage of *sn^{vw}* is

that the transcription of the KP element will probably be governed by the *singed* promoter and enhancers, and the transcription pattern of *singed* is known (Paterson & O'Hare, 1991). *singed* is transcribed in the pupae in both males and females, but in larvae is transcribed only in ovaries. This would suggest that if the KP polypeptide product acts as a repressor, the rate of reversion of sn^{vw} in homozygous females might be lower than the rate in hemizygous males under the same conditions. However, there might be many reasons why reversion rates in females and males could be different, quite apart from any effect of a KP protein produced in females. The number of cell generations in the female germ line will be fewer than in the male germ line. Conversely, the transcription of *singed* may well make the gene, and the P elements within it, more accessible to P proteins, and create a higher reversion rate. This predicted difference in rate would be independent of the particular structure of elements located at *singed*.

There is, however, an experiment in which the effect of female-specific transcription of *singed* on the reversion rate of sn^{vw} can be studied. sn^{vw} males can be produced in attached-X crosses, in which they receive the mutation from their fathers. They can also be produced by standard crosses in which they receive the mutation from their mothers. Mothers possessing *Mo* can be used in each cross, but the difference is that in the latter there will also be transcription of the KP element in the maternal germline. If this transcription of KP from sn^{vw} has an effect on the power of *Mo*, this could be observed through a change in the mosaicism rate of sn^{vw} in the F$_1$ males. It is possible to carry out this experiment because, in keeping the sn^{vw} mutation on the hemizygous X chromosome in males kept with attached-X females, a rare nondisjunction event made it possible to establish a line in which the females were homozygous for the sn^{vw} mutation. Nevertheless, this strain was, apart from the X chromosomes, genetically identical to the attached-X strain from which it was derived. By comparison of the rates of somatic excision of P elements from sn^{vw} in crosses using these two strains, it is possible to see the maternal effect of the sn^{vw} allele itself on sn^{vw} mosaicism in F$_1$ males.

Materials and methods

Drosophila melanogaster *strains*

Strain 1. C(1) DX*yf*/T(1:Y)*y*$^+$; *pr b dp*; *st* ♀s and *y* sn^{vw}; T(1:Y) *y*$^+$; *pr b dp*; *st* ♂s. This is an M' strain

that initially lacked P elements except for the male X chromosome, which has two deleted P elements inserted in sn^{vw}. We have since discovered (P. Emery, T. Robinson and J. F. Y. Brookfield, unpublished data) that the strain possesses a single form of short P element (of around 400 base pairs) which is located on both second and third chromosomes. This may have been derived by low level movements from the X to the autosomes in males during the 4 years during which this strain has been kept. Nevertheless, the sn^{vw} mutation has remained entirely stable in this strain. For a full account of the production of this strain see Ortori *et al.* (1994) where it is referred to as strain 6.

Strain 2. This strain is as strain 1 but has second chromosomes heterozygous for *Mo/pr b dp*. This strain was derived by crossing attached-X *Mo* females to strain 1 males above. F$_1$ *pr*$^+$ *b*$^+$ *dp*$^+$ *Mo/pr b dp* females were selected, and crossed to further strain 1 males. This crossing scheme has been repeated for more than 20 generations. In this time all material in the original *Mo* strain will have been replaced by strain 1 material, with the exception of the region of the second chromosome between *dp* and *Mo*, and the attached-X chromosomes. However, the attached-X chromosomes in this strain were originally the same as those in strain 1, and lacked P elements. The linkage of *pr*$^+$ to *Mo* allows *pr*$^+$ to act as an approximate visible marker for *Mo*.

Strain 3. *y* sn^{vw}; *pr b dp*; *st* ♀s and *y* sn^{vw}/T(1:Y) *y*$^+$; *pr b dp*; *st* ♂s. The males and females possess the same X chromosome and autosomes. This strain was derived from a nondisjunction in a strain 1 male, in which two X chromosomes entered the same sperm. Fertilization of a Y-bearing oocyte yielded an XXY female which then established a line in which sn^{vw} was present in male and female X chromosomes, but the strain had identical autosomes to those in strain 1.

The three strains shared recent common ancestry very soon before the commencement of the experiment, which implies that the short autosomal P elements subsequently seen in strain 1 would have been present in strains 2 and 3 also.

Crosses

Figure 1 shows the crosses performed. Five strain 2 males were taken individually and crossed to both strain 3 and strain 1 females. From the F$_1$ of the former cross, two crosses were set up. Firstly, *pr*$^+$/*pr*

Fig. 1 Crosses in the experiment. Individual parental males (strain 2) were crossed to females from strain 3 (homozygous sn^{vw}), and females from strain 1 (attached-X with sn^+). F_1 males and females from each cross were selected for pr^+, and thus Mo, and their sons examined. Cross (i) F_2 males show the rate of mosaicism of sn^{vw} when the mother is homozygous for sn^{vw}. The proportion of germline revertants seen in these males shows the reversion rates in their mothers' germlines. Cross (ii) and cross (iv) F_2 males show the rate of reversion in the germlines of F_1 males with zygotic Mo. Cross (iii) F_2 males show the rate of mosaicism in sn^{vw} males with homozygous sn^+ mothers.
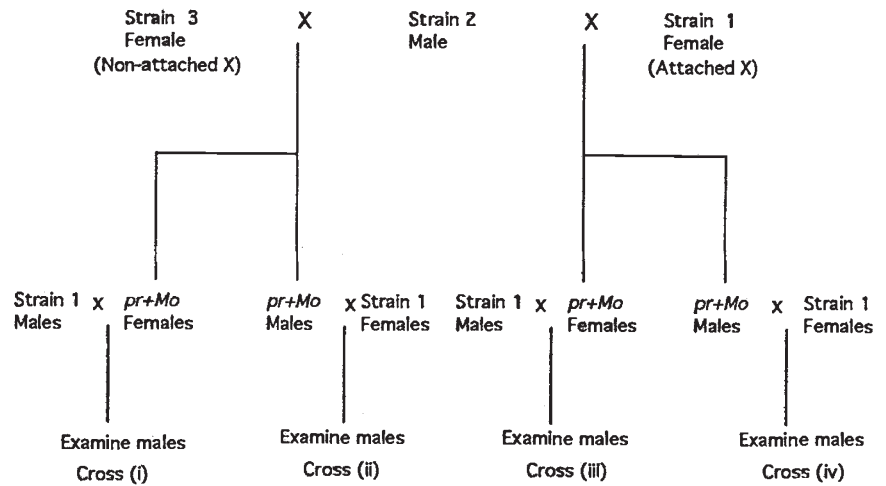
females (which possessed the Mo allele) were crossed to strain 1 males. This is referred to as cross (i) below.

Secondly, pr^+/pr F_1 males were crossed to strain 1 females. This is referred to as cross (ii).

From the second parental cross, to strain 1, two crosses were carried out with F_1 flies. pr^+/pr females were crossed to strain 1 males (cross iii), and F_1 pr^+/pr males were crossed to strain 1 females (cross iv). The purposes of these crosses were as follows.

Cross (i) screens the sn^{vw} offspring of Mo sn^{vw} mothers for mosaicism of sn^{vw}. Furthermore, as F_2 males are derived from gametes from F_1 females, examination of F_2 males measures the germline reversion rate of sn^{vw} in these females, which possessed zygotic Mo but lacked maternal Mo.

Cross (ii) examines the level of germline reversion of sn^{vw} in males in the presence of zygotic but not maternal Mo.

Cross (iii) screens the sn^{vw} offspring of Mo sn^+ mothers for mosaicism of sn^{vw}.

Cross (iv) also examines the level of germline reversion of sn^{vw} in males in the presence of zygotic but not maternal Mo. However, the sn^{vw} allele examined in cross (ii) is derived from strain 3, whereas that examined in cross (iv) is derived from strain 2.

Five to 20 individual crosses were carried out for each cross with each starting male, with the exception of male 1, for whom cross (iv) was not performed. The marker pr^+ will be in coupling with Mo in all F_1 offspring of a given parental male, as no recombination will occur in these males. Thus, if all parental males possess Mo, Mo will be found in all F_1 flies selected though their possession of pr^+. The

individual males examined in the F_2 will consist of a mixture of flies heterozygous for Mo and homozygous for its absence. However, Mo is not required zygotically for mosaicism and the distribution of Mo among males in the F_2 will be the same for crosses (i) to (iv).

Flies were maintained at 22°C in a 12 h light:12 h dark cycle on a standard molasses and maize meal mixture.

Results

Mosaicism was found in the F_2s of crosses (i) and (iii) for crosses using all five parental males, indicating that all these five males indeed possessed Mo. Thus all F_1 flies selected using pr^+ would also possess Mo. Male offspring from the four crosses (i), (ii), (iii) and (iv) can be divided into four classes. These are sn^{vw} mosaics, sn^{vw} nonmosaics, sn^e, and sn^+. The numbers in each class are shown in Table 1.

The clearest result is the consistent difference in mosaicism rates between crosses (i) and (iii). The males examined for mosaicism of sn^{vw} in these crosses have the same zygotic genotypes, but differ in that, while their mothers all possess Mo, in cross (i) the mothers have sn^{vw} whereas, in cross (iii), the mothers have the sn^+ allele on their attached-X chromosomes. The average mosaicism rate in cross (i) is 10.03 per cent (based on 1575 sn^{vw} males) whereas in cross (iii) it is 33.6 per cent (based on 631 males). In five repeats out of five the difference goes in the same direction (which is itself significant at the 5 per cent level in a one-tailed test). Within

each of the five repetitions the proportions of mosaics in crosses (i) and (iii) are very significantly different in G-tests. However, in this experiment, as in earlier ones with this system, females which all possess *Mo* nevertheless show significant variability in the proportions of mosaics in their male offspring.

Table 1 Phenotypes of F_2 males

Cross number	Mosaic sn^{vw} males	Nonmosaic sn^{vw} males	sn^e males	sn^+ males
Male 1				
(i)	32	207	2	2
(ii)	0	370	4	18
(iii)	58	55	0	0
Male 2				
(i)	38	230	0	35
(ii)	0	360	7	7
(iii)	58	79	0	0
(iv)*	0	167	2	5
Male 3				
(i)	46	555	0	6
(ii)†	2	766	12	2
(iii)	52	181	0	0
(iv)	0	365	5	4
Male 4				
(i)	8	153	15	4
(ii)	0	301	5	5
(iii)	19	46	0	0
(iv)‡	0	114	4	1
Male 5				
(i)	34	272	2	1
(ii)	0	180	2	8
(iii)	25	58	0	0
(iv)	1	198	2	0

*Also contains one male more extreme *singed* than sn^e.
†Also contains 2 males more extreme *singed* than sn^e, plus one male more extreme than sn^{vw} but less extreme than sn^e.
‡Also contains one *forked* male (caused by breaking of the mother's attached-X *yellow forked* chromosome).

This means that individual F_2 males are not independent samples. To overcome this problem, I have calculated the proportion of mosaics in the sons of F_1 females in crosses (i) and (iii), and compared the females using the nonparametric Mann–Whitney U-test. This test takes all females in crosses (i) and (iii) and ranks them according to the proportions of mosaic sons which they produce. The null hypothesis is that females from the crosses (i) and (iii) are randomly distributed with respect to this ranking. The results of the Mann–Whitney test are given in Table 2.

These tests reveal significance more mosaics in cross (iii) for males 1, 2, 4 and 5. For male 3 the data diverge from the null hypothesis in the same direction, but narrowly miss significance ($P = 0.054$). With respect to male 3 two females in cross (iii), surprisingly, show no mosaic males in their offspring, despite the large number of males examined (42 and 44, respectively). This is surprising as the lack of recombination in males implies that these females should, like their sisters, have possessed *Mo*. This may be a sign of male recombination in the region, separating *Mo* from pr^+ (although recent experiments looking for male recombination in the presence of *Mo* gave rates under 0.1 per cent (J. F. Y. Brookfield, *et al.*, unpublished data)). Alternatively, it could be a sign of instability of the *Mo* mutation itself.

The data for germline reversion in males and females in Table 1 give no clear evidence for a reduction in the rate of reversion in females in cross (i) relative to males in crosses (ii) and (iv). The similarity of rates in crosses (ii) and (iv) gives reassurance that the sn^{vw} in strains 1 and 2 (being tested in (i), (iii) and (iv)) behaves exactly the same as that in strain 3 (being tested in (i) and (ii)). There is a problem with these data, however. The reversions of sn^{vw} to sn^e and sn^+ products are clustered in males but much more strongly clustered in females. For male 2, for example, there are a very large number of pseudo-wild-type F_2 males in cross (i). Of

Table 2 Mann–Whitney U-tests to compare mosaicism in crosses (i) and (iii)

Male number	F_1 females tested–cross (i)	F_1 females tested–cross (iii)	Value of test statistic U	Probability (two-tailed)
1	9	7	63	0.0010
2	19	7	116	0.0045
3	20	7	105	0.0541
4	16	6	84.5	0.0021
5	17	8	102.5	0.0471

these, 15 males are from a single female, who only produced 13 unreverted sn^{vw} male offspring. The simplest explanation is that this female was actually heterozygous for sn^{vw} and its sn^+ product, having received a sn^+ allele from her father (who could have had reversions in his germline). The problem is that such heterozygotes (and indeed sn^{vw}/sn^e heterozygotes), cannot be reliably distinguished from sn^{vw} homozygotes. This means that the observed sn^{vw} reversion rate in these females is actually the sum of their reversion rates and those of their sn^{vw} fathers. Similarly, the fourth male gave many sn^e F_2 males in cross (i), but in one case six of 16 F_2 males were sn^e, and in another case four of seven were. Again the F_1 females tested were probably heterozygotes.

To overcome these artefacts, I examined all the offspring in cross (i), and scored only the numbers of sn^{vw} and revertant males for all females where the proportions of revertants (of each of the two revertant classes) were significantly less than half. This was designed to eliminate cases where the mother was not homozygous for sn^{vw}. Clearly, this procedure will introduce a downward bias in the reversion rate, and so the same procedure was applied to the results of crosses (ii) and (iv), so that the data remained comparable. The results of this procedure are given in Table 3.

If, in the adjusted data, crosses (ii) and (iv) are pooled, and the average compared with the cross (i) results, the total rate of reversion in males is 50 per cent higher than in females (3.3 per cent vs. 2.2 per cent). However, it is impossible to treat individual F_2 revertant males as independent observations, as they occur in clusters derived from single reversion events in their mothers or fathers. No significant evidence for a reduced rate in females comes from these data. The rates in males are broadly similar to those seen earlier (5.5 per cent total reversion rate

in the presence of zygotic but not maternal Mo (J. F. Y. Brookfield *et al.*, unpublished data).

Three mosaic males were observed in crosses (ii) and (iv), which are difficult to explain as these sn^{vw} males would not have had Mo mothers. If they do not represent errors in scoring then they may indicate a low level of somatic reversion resulting from a zygotic effect of the Mo allele (as all three males had this).

Discussion

There is a very clear and significant reduction in the level of mosaicism of sn^{vw} in cross (i) relative to cross (iii). The males that are being examined for mosaicism in these crosses have the same genotype and differ only in the genotypes of their mothers. The threefold reduction in mosaicism rate observed must, therefore, be caused by the substitution of the sn^{vw}-bearing X chromosome for the attached-X chromosome. The sn^{vw} chromosome was originally from Canton S, a pure M strain, and thus lacked P elements (Brookfield & Mitchell, 1985). We have no knowledge of any P elements on this chromosome other than the two at sn^{vw}. It seems probable that the attenuation of mosaicism in the F_1 results from the effects of these P elements at *singed*. One could imagine that this effect is a form of transposase titration (Simmons & Bucholz, 1985; Simmons *et al.*, 1987, 1990), in which it is the binding, in the mother, of a P product from Mo to the X-linked P elements that makes this product unavailable for incorporation into the egg, and thus reduces the level of excision in the zygote. Two arguments can be raised against such a transposase titration model. First, the titration of the transposase that will make it unavailable to destabilize sn^{vw} does not happen simultaneously with sn^{vw} reversion. The sn^{vw} allele in the

Table 3 Reversion rates in males and females. (a) Proportions of revertants in crosses (i), (ii) and (iv) pooled across all five original males. (b) Proportions of revertants in crosses (i), (ii) and (iv) following adjustment

Cross	sn^{vw} males	sn^e males	sn^+ males	Other revertants	Total reversion rate (%)
(a)					
(i)	1575	19	48	0	4.1
(ii)	1979	30	40	3	3.6
(iv)	785	13	10	1	3.0
(b)					
(i)	1486	4	30	0	2.2
(ii)	1948	27	40	3	3.5
(iv)	762	13	10	1	3.1

mother to which the transposase from *Mo* is hypothesized to bind will itself be passed into the oocyte, and thus, if the transposase is subsequently released, this binding will not reduce the availability of the *Mo* product for the zygotic excision process. Secondly, the size of the mosaic patches in the sons reveals that the excisions do not commence at fertilization, but after a number of cell divisions (Brookfield & Lewis, 1989), when there are hundreds of nuclei. It is hard to imagine the two copies of sn^{vw} in the mother significantly reducing, by a titration effect, the transposase available to the hundreds of sn^{vw} copies in the F_1 at the time of destabilization.

The data seem more consistent with the idea that KP transcription, which results from the element's presence in *singed*, reduces in some way the effective level of *Mo* product in the oocyte. This could be by reducing the level of *Mo* put into the egg, perhaps by an effect on *Mo* transcription, or by including a KP product in the egg that antagonizes the *Mo* product in the zygotic nuclei.

There is, however, nothing in this experiment that demonstrates that the level of repression induced by the KP element is any higher than that induced by other deleted P elements of similar or greater size. Indeed, perhaps the most obvious characteristic of the repression is that it still allows 10.03 per cent of offspring to be visibly mosaic. Indeed, there is no demonstration that it is the KP element in sn^{vw} that is generating the repression. Rasmusson *et al.* (1993) found that a very short P element, SP, of 517 base pairs, was capable of eliciting a strong repression of sn^w reversion and of gonadal dysgenesis, despite the fact that the predicted polypeptide encoded would contain only 14 amino acids. These authors suggest that the SP sequence could repress through the formation of an antisense P RNA by way of read-through from an external promoter. Interestingly, sn^{vw} also has a short element which would be transcribed from the wrong strand by transcription initiating at the *singed* promoter. Nevertheless, in view of the earlier observation that KP may be capable of repression in other systems, and of the absence of any evidence of such repression arising from the other element in sn^{vw}, the most parsimonious explanation for the effect noted here is an effect of KP itself.

Acknowledgements

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