Isolation and characterization of the *zeste* locus of *Drosophila*

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The *zeste* gene of *Drosophila* regulates the expression of certain other genes like *white*, *bithorax* and *decapentaplegic* in a manner dependent on chromosome pairing. The genetic evidence suggests that its product interacts with the genes at the level of transcription. We have cloned the *zeste* region from fragments microdissected from the 3A1-4 region of the X chromosome and mapped the breakpoints of several rearrangements which localise the gene. We have isolated dysgenic *zeste* mutants which contain P element sequences inserted in the same region. DNA rearrangements were found in some *zeste* mutants but were not detected in the *z*¹, *z*^{op6} or *z*^{op11} mutants. The changes are localised to a *Bam* 4.0-kb fragment from which originates a 2.4-kb RNA species. This transcript is altered in some *zeste* mutants but is not visibly affected in *z*¹ or *z*^{op6} mutants. The region bears no homology to the *white* gene or its vicinity but cross-hybridises to many other genomic sequences in *Drosophila*.

Key words: chromosome pairing/gene regulation/transvection/*zeste* gene

Introduction

Genes that exert a regulatory function on the expression of other genes are well known in bacteria and yeast and have been postulated on the basis of genetic evidence in other eukaryotes. Genetic evidence tells us that the *zeste* (*z*, X-1.0) gene of *Drosophila* is a regulatory gene which affects the expression of at least three other loci: *bithorax* (*BX-C*), *decapentaplegic* (*dpp*) and *white* (*w*). It is unique in that its regulatory activities depend upon the pairing of two alleles of the target loci in somatic cells. This unusual type of interaction has attracted many investigators since its initial discovery by Gans (1953). Their combined efforts in the past 40 years have accumulated a mass of observations which suggest the existence of transcriptional regulatory mechanisms that are affected by the proximity of sequences on other DNA molecules.

The product of the *zeste* gene interacts with *BX-C*, *dpp* and *w* and causes an apparent enhancement of the expression of these loci (Gelbart, 1982; Gelbart and Wu, 1982). This effect is noticeable by the ability of certain mutant alleles of these loci to complement partially one another in the presence of an intact *zeste* gene. Thus, the phenotypic effects of certain combinations of *BX-C* alleles are alleviated in the presence of *z*⁺ but accentuated by *zeste* mutations (Kaufman *et al.*, 1973). In the *white* locus, the *w*^{sp} mutation (which affects the regulatory region of

the gene) can be partially complemented by other *white* alleles such as *w*^a (which affects the gene but not its regulatory region) in the presence of *z*⁺ (Babu and Bhat, 1980). The unusual aspect of the *zeste* interaction is that it is enhanced by the presence of two paired copies of the target gene (Lewis, 1954; Jack and Judd, 1979). Thus, chromosomal rearrangements which prevent the somatic pairing of the two copies of the *BX-C* locus present in the two homologous chromosomes prevent the *zeste* effect just as mutations in *zeste* would (Lewis, 1959; Kaufman *et al.*, 1973). This synergistic interaction of two paired copies of the locus was termed transvection by Lewis, and applies to all three of the loci which have been shown so far to interact with *zeste*. Much of the information on the *zeste* effect comes from the interaction of one particular *zeste* mutation with the *white* locus. This is *z*¹, a spontaneously arising allele isolated and analysed by Gans (1953). This mutant has an altered *zeste* gene whose product is still able to interact with the target genes but with the opposite effect: instead of enhancing the expression of *white* or of *dpp*, it depresses it. With respect to *BX-C*, Kaufman *et al.* (1973) and Gelbart and Wu (1982) have shown that the *z*¹ product behaves like the normal product.

The influence of chromosome pairing is strikingly illustrated by the interaction of *z*¹ with *w*. Since *w* is on the X chromosome, males have a single copy of the gene, while females have two, paired copies. In the presence of *z*¹ the expression of *w* is strongly reduced in females resulting in yellow eyes, while *z*¹ males have red eyes. The target of the *z* product is the regulatory part of the *w* locus. Mutations in this region, such as *w*^{sp}, fail to interact with *z*¹ (Green, 1959; Judd, 1959; Kaufman *et al.*, 1973). Tandem duplications of this region suffice to enhance the *zeste* effect on a single chromosome (Judd, 1961; Green, 1963; Jack and Judd, 1979). Starting from the *z*¹ mutant, Lifschytz and Green (1984) have isolated double mutants in the *z* locus which formally behave as overproducers of *z*¹ and give a range of phenotypic effects. The stronger mutants (e.g., *z*^{op6}) now give effects even with a single copy of *w*.

Most of the mutants in the *zeste* locus are formally equivalent to reduction or loss of function and are referred to as *z*^a type. Mutants of the *z*¹ type are much rarer than the *z*^a type, and correspond formally to an altered function.

The genetic results imply: (i) that the *zeste* product has its effect either on the DNA itself or on transcription but not on translation or post-translational processes, (ii) that *zeste* can act also on single copies of the target gene (as shown by the *z*^{op6} mutation) but that the vicinity of more copies of the target gene enhances the effect; (iii) the effect of the *z*¹ product appears to be antagonistic to that of the *z*⁺ product. Although *z*¹/*z*⁺ females have apparently normal expression of *w*, the *z*¹ effect becomes gradually visible with increasing numbers of tandemly repeated *w* genes (Jack and Judd, 1979). Similarly, *z*^{op6} which behaves as an overproducer of *z*¹, is dominant over *z*⁺ in the presence of two paired copies of *w* (Lifschytz and Green, 1984). Analysis of the *white* RA from *z*¹ male and female adult flies or from *w*^{sp} flies showed that, in spite of the drastic reduction in pigmen-

tion, there is no corresponding reduction in the overall transcription of the *white* gene in these mutants (Pirrotta and Bröckl, 1984; O'Hare *et al.*, 1983). Since both *zeste* and *w^{sp}* affect the *white* regulatory region and not the coding region, these findings imply that the defect lies in the time or tissue specificity of *white* expression.

The genetic data give us no inkling of the normal function of the *zeste* gene. It may not be an essential function although this has not been strictly established since *z^a* mutants may have residual activity. The molecular details of *z^a* action are likely to involve questions of chromosome structure and interactions, chromatin structure and promoter regulation. As a first step in the study of the molecular mechanisms of *zeste* activity, we report here the cloning of the *zeste* locus and some of its properties.

We used two independent approaches to isolate *zeste*. One was to gain entry to the *zeste* region by microdissection and microcloning (Scalenghe *et al.*, 1981; Pirrotta *et al.*, 1983b) of the chromosomal region in which *zeste* has been mapped. The second was to induce insertional mutations in the *zeste* locus by a P-M dysgenic cross (Rubin *et al.*, 1982) and then use the P transposable element as a probe to recover the sequences flanking the P insertion site.

Results

Microcloning of the *zeste* region

Gans (1953) assigned the *zeste* locus to band 3A3 on the X chromosome on the basis of deletion mapping with *Df(1)s²⁵⁸⁻¹¹* and *Df(1)w²⁵⁸⁻¹⁴*. This assignment was confirmed by Judd *et al.* (1972) using additional deletion breakpoints.

This region of the X chromosome contains two prominent and closely spaced band doublets: the 3A1,2 and 3A3,4 doublets. We used the microdissection technique to cut chromosome fragments from the middle of the frequently unresolved 3A1-4 complex.

From three microdissected chromosome fragments, the microcloning technique yielded 31 clones containing *EcoRI* fragments ranging from <0.5 to 8 kb. We screened these clones by hybridization with total, nick translated *Drosophila* DNA to eliminate those containing repetitive sequences and used the remaining clones as probes to screen cosmid and phage libraries. Our initial findings could be assembled in two clusters of clones, nucleated around cosmids T6, T16 and T17 on the one hand and T1 and T4 on the other. We tested these clones by *in situ* hybridization to polytene chromosomes containing the *e(bx)* muta-

Fig. 1. Map of the region surrounding the *zeste* locus. The coordinates are marked in kilobases using the insertion site of the P element in the *z^π* mutant as the origin. Representatives of the clones used to construct the map are shown below each map segment. The breakpoints of *In(1) e(bx)*, *Df(1) 64C4*, *Df(1) w²⁵⁸⁻¹¹*, *Df(1) wrj1* and *Df(1) 62g18* are shown with the solid line indicating DNA present. The insertion at position +17 is found in several strains and is present as a polymorphism in our population of Oregon R. The insertion at position +5 is found in the *z^π* stock and the P insertions found in the *z^π* mutant are indicated at positions -69 and 0. Some restriction site polymorphisms are also shown: H* at -8.0 is found in *z^π* and derivatives, *z^α*, *z^{π77h}* and Canton S; H* at -3.0 is found in Oregon R, *z^π* and derivatives only; R* at +2.0 is missing in *z^π* and derivatives. The cluster of Rs marked with * at +22 indicates that a variable number of tandemly repetitive units of 0.2 kb, containing an *EcoRI* site is found in different strains at this position. R: *EcoRI*, H: *HindIII*, B: *BamHI*.

tion. This mutation, isolated by Lewis (1959) is associated with a small inversion with breakpoints in 3A3 and 4F. Kaufman *et al.* (1973) showed that it behaves as a z^a mutation, strongly sug-

Fig. 2. *In situ* hybridisation to *In(1) e(bx)* chromosomes. The DNA of clone λ T17-6 was labelled with [35 S] α -thio-nucleotide triphosphates and hybridised to *In(1) e(bx)* salivary gland chromosomes. Heavy hybridisation to both ends of the *In(1) e(bx)* inversion shows that the clone contains approximately equal amounts of DNA on each side of the breakpoint.

gesting that the 3A3 breakpoint is in or very near the *zeste* locus. The *in situ* hybridisation showed that the cos T1–T4 cluster is distal to the 3A3, while the cos T6–T17 cluster is proximal. We then extended the cloned region by chromosome walking to obtain a single continuous set of clones represented in Figure 1.

The restriction map of the region connecting cosT17 to cosT1 shows that it contains very few *Eco*RI sites and explains why it was not represented in the microdissection clones, which are limited by the capacity of the vector to *Eco*RI inserts <10–11 kb. Using clones from this region we were now able to map the site of the *e(bx)* breakpoint. Figure 2 shows that clone T17-6 spans this breakpoint and hybridises *in situ* to both sides of the inversion. A more precise position for this breakpoint and that of several deletions affecting this region were obtained by genomic Southern blot hybridisation. Figure 3 shows that both *e(bx)* and *Df(1)64c4*, which deletes 3C2-3 and 3A3-4 and lacks the *z* locus, have their distal breakpoint between position –3.5 and +0.5 in the map shown in Figure 1. Genomic S1 mapping experiments (not shown) confirm that these two breakpoints are within 200 bp of each other, near position –1. *Df(1)w²⁵⁸⁻¹¹*, which is very similar to *64c4* and is also deficient for *z*, has a breakpoint between positions +1 and +3. *Df(1)s^{1/1}*, which starts in the *w* locus and includes both *z* and *tko*, the next distal marker, breaks at 3A1-2. We found this breakpoint between positions +3 and +6 in our map, implying that *tko* is bracketed between positions +1 and +6. *Df(1)62g18* includes *z*, *tko* and the *giant* locus (*gt*) and has a breakpoint around position +63 to 64. We

Fig. 3. Genomic Southern blot hybridisations of chromosome rearrangements. **Panel A** shows (1) Canton S DNA, (2) *e(bx)* DNA, (3) Oregon R DNA, cut with *Bam*HI and hybridised with the *Bam* 4.0 probe. **Panel B, C and D** show (1) *Df(1) w²⁵⁸⁻¹¹/w⁺Y* DNA, (2) *Df(1) 64C4/w⁺Y* DNA, (3) *Df(1) w¹/w⁺Y* DNA cut with *Eco*RI and hybridised with the *Bam* 4.0 probe (**panel B**), with the *Bam* 2.4 probe (**panel C**) with the *Bam* 3.2 probe (**panel D**). A new band, not found in the *w⁺Y* balancer chromosome appears if the probe hybridises to the restriction fragment containing the breakpoint. The map below shows the origin of the three probes used.

were able to map unambiguously the breakpoint of *Df(1)K95* (3B1-2 to 3A3-4) which does not include *z*. The genomic Southern blots (not shown) indicate that it is very likely between positions -25 and -15 but contains a complex rearrangement with respect to the Oregon R and Canton S wild-type strains which precluded a more precise localisation. A rearrangement was also found at the proximal breakpoint of *K95*, as reported by Reddy *et al.* (1984).

These results indicate that at least part of the *z* gene is contained between position -25 and -1. The *e(bx)* breakpoint strongly suggests that the gene is in the close vicinity of position -1.

Dysgenic *zeste* mutants

Our second approach to identify and isolate the *zeste* gene was to generate dysgenic *zeste* mutants by crossing wild-type Oregon R females with males from the $\pi 2$ strain of Engels and Preston (1981). As indicated by the crossing scheme in Figure 4A, the progeny of this cross was then mated with corresponding partners from a stock of *In(1)64b9 y² z spl* to reveal *zeste* mutations. From ~50 000 chromosomes, we found five independent mutants which gave a *zeste* phenotype in heterozygosity with the *y² z spl* chromosome. Three of these we found later to be extensive deletions which were homozygous lethal and not further investigated. The remaining two, z^π and $z^{\pi 2}$, were homozygous viable and contained insertions of P element sequences in the 3A1-4 region, as shown by *in situ* hybridisation to polytene chromosomes (Figure 5). The parent $\pi 2$ strain gives no hybridisation to a P probe in this region. When heterozygous with the z^1 mutation, z^π gives a yellow eye color while homozygous z^π/z^π flies have red eyes. In the *zeste* terminology, this means that z^π is a z^d or loss of function mutation. However, upon closer inspection, homozygous z^π females, but not males, frequently have a slightly lighter eye color with distinctly uneven pigment distribution, as if heavily spotted. This female specific effect, though very weak, is reminiscent of the phenotype of z^1 flies raised at 18°C. The $z^{\pi 2}$ mutation when heterozygous with z^1 gives a yellow-orange eye color indicative of incomplete loss of function. Homozygous $z^{\pi 2}$ flies have wild-type eyes.

Revertants of z^π were isolated by a second dysgenic cross screened according to the scheme in Figure 4B. About 1 in 300 females of the progeny from this crossing scheme is at least a partial revertant with eye colors ranging from dark brown to red.

Cloning the z^π insertion mutant

The *in situ* hybridization to z^π polytene chromosomes showed, as expected, the presence of many other P elements scattered throughout the chromosome arms. To isolate genomic clones containing the P insertion in the *zeste* locus, we resorted again to the microdissection technique. From 10 chromosome fragments isolated from the 3A1-4 region of the z^π mutant, we obtained ~500 recombinant clones which were screened directly for the presence of P sequences. Four clones hybridised to a P probe, three were identical (type $\pi 1.4$), the fourth was different (type $\pi 11.5$). When these two types of clones were tested by hybridisation against an array of clones representing the entire region shown in Figure 1, we found that type $\pi 1.4$ came from the vicinity of position -70 while clone $\pi 11.5$ came from the distal end of cosT17, 70 kb away. The restriction maps of the two types of clones correspond to the regions indicated in Figure 1. We concluded that in the dysgenic cross two insertional events had occurred at a distance of 70 kb, only one of which, presumably, was responsible for the *zeste* mutation.

The proximity of the $\pi 11.5$ insertion site to the breakpoint

Fig. 4. (A) Crossing scheme for the generation of dysgenic *zeste* mutants. Oregon R virgins were crossed with males from the P-containing $\pi 2$ strain of Engels and Preston (1979). Male and female offspring were crossed separately to partners from the *In(1)64b9 y² z spl* stock. The progeny was screened for females showing the *zeste* eye phenotype. (B) Crossing scheme to generate revertants of z^π . Male z^π which had been maintained in a stock with *C(1)DX, yf; pi2* females, were crossed to females *C(1)DX, w, cv* to induce dysgenesis. The male progeny were crossed to females *In(1)64b9 y² z spl* and the resulting females screened for eye phenotypes approaching the wild-type.

Fig. 5. In situ hybridisation to z^π chromosomes. Salivary gland squashes from z^π larvae were hybridised to a ³H-labelled $\pi 25.1$ plasmid containing a 2.9-kb P element and flanking sequences from the 17D region (Spradling and Rubin, 1982). Several hybridising sites are visible on the X chromosome. The arrow points to the 3A1-4 region where *zeste* is located.

of *e(bx)* strongly suggested that this and not the $\pi 1.4$ insertion was responsible for the z^π mutation. We therefore chose the P insertion site in $\pi 11.5$ as the coordinate origin of the map shown in Figure 1.

Analysis of *zeste* mutants

To confirm the identification of the region around position 0 as the *zeste* locus, we hybridised genomic Southern blots of several *zeste* mutants to probes derived from clone T17-6. Figure 6 shows that the z^π mutant has an insertion of apparently 0.7 kb at position 0, in agreement with the restriction map of clone $\pi 11.5$. This mutant also has an insertion near position -70, in agreement with the restriction map of clone $\pi 1.4$. A revertant of the z^π mutant ($z^{\pi r}$) shows that the *Bam* 4.0-kb fragment containing the insertion at position 0 has become smaller, indicating that it has suffered extensive deletion. In contrast, the insertion at position -70 is unchanged, confirming the identification of the $\pi 11.5$ insertion as the one responsible for the mutant phenotype. The second dysgenic mutant, $z^{\pi 2}$ also has an insertion at or very near the same site as the $\pi 1.5$ insertion, but ~1.5 kb larger. Unlike the z^π mutant, $z^{\pi 2}$ has no insertion at position -70.

Fig. 6. Genomic Southern blots of *z* mutants. Genomic DNA from the strains indicated was cut with a suitable restriction enzyme, electrophoresed and blotted onto nitrocellulose filters. The DNA in **panel A** was cut with *Bam*HI and the filter hybridised to the *Bam*HI 4.0-kb fragment that includes the coordinate origin. **Panel B** shows a collection of mutant DNAs cut with *Bam*HI and hybridised with clone λ T17-6. z^{81E} is a recombinant between z^1 and z^{v77h} , isolated by M.Green. **Panel C** shows that the reversion of z^π in the $z^\pi r$ revertant is accompanied by a deletion. The DNA was cut with *Bam*HI and hybridised with λ T17-6. In **panel D**, the DNAs were cut with *Eco*RI and hybridised with probe λ 21.2, showing that the P insertion at the -69 site is not affected by the reversion. The $z^\pi r$ chromosome used in this case was heterozygous with the $y^2 z spl$ chromosome.

The region around the *z* locus is rich in a variety of polymorphisms which complicate the analysis of mutant DNA. Some of these polymorphisms are most likely simple, local sequence variations. They are detected by the presence of additional restriction sites or the lack of restriction sites found in other strains without detectable changes in the pattern produced by other restriction enzymes. For example, the *Hind*III site at position -5 in the Oregon R strain is missing in Canton S, z^a , z^{v77h} and in the π_2 strain and derivatives, while Canton S, z^1 , z^a , z^{v77h} , but not π_2 have an additional *Hind*III site at position -8. Similarly, the *Eco*RI site at position +1.2 is missing in z^1 and all its derivatives. It is unlikely that these differences are responsible for any of the mutant phenotypes.

Some polymorphisms are insertional. The Canton S strain, z^a and a fraction of our Oregon R population contain a 2-kb insertion at position +18. In addition, the DNA of the z^a mutant contains an insertion of ~5 kb at position +6. We cannot determine at this point whether the mutant phenotype is caused by this insertion or by other, subtler changes.

The z^1 mutation has a neomorphic phenotype: it does not behave like a loss of function but like an alteration which causes it to become an antagonist of normal *zeste* function. A partial revertant of z^1 called z^{11G3} was isolated by Gans (1953) after X-ray treatment. For these reasons, z^1 has frequently been supposed to be due to an insertion or similar DNA rearrangement. We hybridised genomic Southern blots of z^1 , z^{11G3} , z^{op6} , z^{op11} and z^{RN4} with a series of clones spanning 60 kb in the proximal direction and 35 kb in the distal direction from the coordinate origin without detecting any difference between these mutants or significant differences with respect to the wild-type strains (not shown). The z^{op6} and z^{op11} mutants are two distinct 'overproducer' mutants isolated from z^1 by Lifschytz and Green (1984), while z^{RN4} is a partial revertant of z^{op6} . The only difference we detected

between these mutants was in the *Bam* 2.4 fragment at position +22. In these mutants, but also in Canton S and Oregon R, Southern blots hybridised with the *Bam* 2.4 fragment give two major hybridising bands whose size varies from strain to strain (not shown). A careful map of this region showed that, in Oregon R, it contains a series of 5-6 *Eco*RI fragments of ~200 bp. Acrylamide gel electrophoresis showed that they are identical in size and that, furthermore, an identical set of fragments is produced by *Hae*II digestion, indicating that they are tandem repeats of a single sequence. We conclude that the differences found in the mutant strains are most likely attributable to variations in the number of tandem repeats at this site and almost surely have nothing to do with the mutant phenotype.

Finally, we analyzed z^{v77} , a mutant isolated by Green (1984) from a dysgenic cross with an MR strain (a male recombination strain carrying a P element). This mutant produces a brown, variegated eye color in both males and females and, when heterozygous with z^1 , behaves like a z^a mutant. The genomic Southern blot in Figure 6 shows that z^{v77h} contains a small deletion of ~300 bp in the 4-kb *Bam* fragment at position -3.3 to +0.7. Genomic S1 mapping experiments (not shown) indicate that the deletion is centered around position 0.

Transcription of the zeste region

Several RNA species come from the region surrounding position 0. Figure 7 shows a Northern blot of poly(A)⁺ RNA from wild-type and several *zeste* mutants, hybridised with the DNA of λ T17-6 (see map in Figure 1). At least three wild-type RNA species hybridise with this probe: a 4.2-kb, a 2.4-kb and a 0.9-kb species. The 2.4-kb species is reduced to 1.8 kb in *e(bx)*, to 2.1 kb in z^π , while in z^{v77h} it is both shortened to 2.1 kb and is of much lower intensity. No size changes are detectable with z^1 or z^a RNA although some changes in the relative intensity of

Fig. 7. Northern blot hybridisation. Approximately 5 µg of poly(A)⁺ RNA from the stocks indicated were separated by formaldehyde agarose gel electrophoresis, blotted onto a nitrocellulose filter and hybridised to the λT17-6 probe. The mol. wts. were estimated using *copia* and *Adh* RNAs as markers.

Fig. 8. Genomic Southern blots of *Drosophila* and other organisms. Genomic DNAs were cut with *EcoRI*, electrophoresed and blotted onto a nitrocellulose filter. (a) 4 µg *Drosophila* DNA, (b) 2 µg yeast DNA, (c) 10 µg mouse BALB/c DNA, (d) 20 µg DNA from a mouse tissue culture line. The filter was hybridised to a 2-kb *HindIII* fragment from position -1.7 to +0.3 in 4 x SSC, then washed extensively in 2 x SSC, 67°C and exposed (left panel). After exposure, the filter was washed again in 0.1 x SSC, 67°C (right panel).

the bands may be significant. In particular, no differences attributable to overproduction are visible in *z^{op6}* RNA.

Hybridisation with shorter probes indicates that the 2.4-kb RNA comes from the *Bam* 4.0-kb fragment (position -3.3 to +0.7), the 0.9-kb species from the *Bam* 3.2-kb fragment (position +3.1 to +6.3) and the 4.5-kb species hybridises to both the *Bam* 2.4-kb fragment (position +0.7 to +3.1) and the *Bam* 3.2-kb fragment (position +3.1 to +6.3).

We conclude from these results that the 2.4-kb RNA can be attributed to the *zeste* gene while the 4.2-kb or the 0.9-kb species or both are likely candidates for the *tko* gene.

Zeste homologies

The sequences in the *Bam* 4.0-kb fragment which codes for the 2.4-kb RNA have homology to many other genomic sequences. Figure 8 shows a genomic Southern blot hybridised with a 2.0-kb *HindIII* probe isolated from the *Bam* 4.0-kb fragment, which codes for at least 1.5 kb of the 2.4-kb RNA. The hybridisation took place under relatively non-stringent conditions (4 x SSC). The filter was first washed with 2 x SSC, exposed a first time and then washed stringently with 0.1 x SSC before re-exposing. Figure 8 shows that the low stringency hybridisation to a large number of *Drosophila* sequences is not resistant to the stringent wash which leaves only the expected 5.8-kb *EcoRI* band. A number of bands hybridising at low stringency are found also in the yeast and in the mouse genome. Most of these do not resist the stringent wash but two distinct bands in the yeast genome and some faint bands in the mouse genome persist. We do not know whether this hybridisation is spurious or whether it indicates the presence of related functions in other organisms. In contrast, probes from the *zeste* region fail to cross-hybridise to any significant degree with the DNA from or surrounding the *white* locus.

Discussion

The genomic region covered by our chromosomal walk spans >200 kb and includes at least two known loci in addition to *zeste*. At the distal end, the breakpoints of deletions *w^{rJ1}* and *62g18* bracket the *giant* locus. This is a developmental locus lethal alleles of which cause a gap in the embryonic segmentation pattern and the loss of two or more abdominal segments. The breakpoints of deletions *w²⁵⁸⁻¹¹* and *w^{rJ1}* bracket the neurological locus *tko* (Judd *et al.*, 1972) and localise it to a 5.0-kb segment between positions +1.0 and +6.5. The recombination map distance between *z* and *tko* is 0.006 map units, corresponding to 5–10 kb and in good agreement with the 6–8 kb indicated by our map. The genetic distance between *tko* and *gt* is said to be six times greater or 30–50 kb, consistent with our deletion mapping results. We have not yet found genetic markers at the proximal end of the cloned region. The locus nearest to *zeste* in the proximal direction is *l(1)zwl* (Judd *et al.*, 1972), which is said to be 0.074 map units from *tko* or 60–100 kb in the proximal direction, hence probably included in our cloned region.

There are several lines of argument to support the claim that we have cloned and identified the *zeste* locus. The first is based on the mapping of chromosomal breakpoints which bracket the *zeste* locus. These results imply that at least part of the locus must lie proximal to position -1, the breakpoint of *Df(1)64c4*, and more distal than the breakpoint of *Df(1)K95*. The argument is damaged by the difficulty in establishing a precise breakpoint for the *K95* deletion. This deletion is a complex one and is evidently accompanied by additional rearrangements near the breakpoints. Reddy *et al.* (1984) report that the proximal breakpoint of *K95*, which lies in 3B1-2, is preceded by a 30-kb inversion. Genomic Southern blot hybridisation suggests that something similar has occurred at the distal breakpoint with rearrangements affecting the region between position -25 and -15.

A more precise localisation of *zeste* is afforded by the *e(bx)* inversion and three dysgenically induced mutants *z^{v77h}*, *z^{pi}* and *z^{pi2}*. These are all located near position 0 in the map and are all *z^a* type mutations, if we ignore for the moment the slight reduction in pigmentation and the variegation seen in *z^{v77h}* and *z^{pi}*. Both *z^{pi}* and *z^{pi2}* have sequences of the P transposable element inserted at position 0. The finding that *z^{v77h}* has a small deletion around

the same site strongly suggests that all three mutations were caused originally by P element insertion, followed by imprecise excision in the case of z^{v77h} . The causal relationship between the P element inserted at this site and the mutant phenotype is confirmed further by the finding that a revertant of z^π has suffered a deletion in the *Bam* 4.0-kb fragment containing the insertion site which has removed both P and flanking sequences. Furthermore, the $e(bx)$, z^{v77h} and z^π mutants also alter the size of a poly(A)⁺ RNA of 2.4 kb transcribed from this region. These results allow us to conclude that the region surrounding position 0 is responsible for an activity lacking in z^a mutants and necessary to complement the z^1 mutation.

Our failure to detect alterations or rearrangements in the DNA of the z^1 mutant and its derivatives is perplexing. The z^1 mutation was spontaneous and apparently unique. Ethyl methanesulphonate mutagenesis does not give rise to z^1 -like mutants although weak alleles which may resemble z^1 have been reported (Gans, 1953; Gelbart, 1971; Gelbart and Wu, 1982). For these reasons z^1 has frequently been supposed to be due to a DNA rearrangement, insertion or deletion which modifies the regulatory or coding region of the gene. However, the available evidence does not exclude a point mutation. z^{11G3} , a partial revertant of z^1 induced by X-rays (Gans, 1953) could be explained as a second site mutation within the z^1 gene. More surprising is the fact that z^{op6} which, according to Lifschytz and Green (1984) is unstable upon X-irradiation, reverting to weaker z^1 -like phenotypes at a frequency of 1 in 300, contained no detectable rearrangements. We are led to two possible conclusions: one is that the alterations in z^1 , z^{op6} , etc., are subtler than we expected. They may be, for example, small inversions which do not change the size of the restriction fragment in which they are contained. Alternatively, the z^1 phenotype is due to a second locus, different from the gene involved in the $e(bx)$, z^π or z^{v77h} mutations but interacting with it in an antagonistic way. A two gene hypothesis for *zeste* was proposed by Lifschytz and Green (1984) on the basis of the apparent competition between z^+ and z^1 products.

Although the present data are not conclusive, we prefer the interpretation that there is only one *zeste* gene and that z^1 is a subtler alteration than was anticipated. A P transposon carrying a 6-kb fragment from the region around position 0, constructed from z^{op6} DNA, confers the *zeste* phenotype even in males (Pirrotta and Bozzetti, unpublished experiments) and confirms the single gene interpretation. Mutations like z^{v77h} , which causes decreased eye pigmentation and variegation, and like z^π , which shows a very slight pairing dependent effect (the female has a slightly variegated and less pigmented eye than the male) suggest that they may represent the low end of a range of z^1 -like phenotypic effects.

While the true nature of the z^1 mutation, like that of the *zeste* function, remains obscure, preliminary S1 mapping data indicate that the *zeste* gene is more complex than is suggested by the simple Northern blots and that the answer may be found by unraveling the complexities of the transcriptional pattern.

Materials and methods

Drosophila strains

Oregon R. Heidelberg: wild-type strain.

Canton S: wild-type strain.

z^1 : carries the original *zeste* mutation (Gans, 1953).

In(1)64b9, $y^2 z sp1$: carries the z^1 mutation and an inversion with one breakpoint in *w* and the other in 12B. The proximal part of the *w* locus is triplicated with one functional copy at the normal site and two at 12B (Sorsa *et al.*, 1973).

y^{z^1} : carries the z^1 mutation of Gans (1953).

z^{v77h} : this mutation produces a variegated eye color in males and females and

was isolated from a dysgenic cross with a Male Recombination (MR) strain (Green, 1984).

z^{81E} : obtained by M. Green from a cross between z^1 and z^{v77h} .

sc z^{op6} *ec*: carries the z^{op6} mutation isolated from z^1 which gives a *zeste* phenotype with a single copy of *w* (Lifschytz and Green, 1984).

C(1)DX, *yf/Df(1) 64c4/w⁺Y*: carries the 64c4 deletion 3A3-4; 3C2-3 which does not complement z (Judd *et al.*, 1972).

C(1)DX, *yf/Df(1) K95/w⁺Y*: carries the K95 deletion 3A3-6; 3B1 which complements z (Judd *et al.*, 1972).

C(1)DX, *yf/Df(1) w²⁵⁸⁻¹¹/w⁺Y*: carries the w^{258-11} deletion 3A3-4; 3C2-3 which does not complement z .

C(1)DX, *yf/Df(1) w^{J1}/w⁺Y*: carries the w^{J1} deletion 3A1-2; 3C2-3 which does not complement z or *tko* (Judd *et al.*, 1972).

C(1)DX, *yf/Df(1) 62g18/w⁺Y*: carries the 62g18 deletion 3A1-2; 3A4-6 which does not complement z , *tko* or *gt* (Judd *et al.*, 1972).

In(1) e(bx): carries an inversion 3A3; 4F associated with a z^1 phenotype (Lewis, 1959).

C(1)DX, *yf*: $\pi 2$ contains multiple P transposable elements and has a P cytotype (Engels and Preston, 1981).

Microdissection and microcloning

The procedure followed is essentially that described by Pirrotta *et al.* (1983b) except that the vector used was λ NM1149 (Murray, 1983) which accepts 0–11 kb *EcoRI* or *HindIII* fragments and grows better than the λ NM641 vector previously used.

Genomic libraries

The libraries used in this work were the Oregon R partial *Sau3A* λ EMBL4 library as well as a cosmid library previously described (Pirrotta *et al.*, 1983a). A few clones were also obtained from a Canton S library constructed the same way and one cosmid clone, cosP4 was isolated from a cosmid library constructed in cosP, a P-mediated transposition vector (Steller and Pirrotta, 1985; Haenlin *et al.*, 1985).

Drosophila DNA and RNA

DNA for genomic analysis was extracted from adult flies by gentle homogenisation with a few pestle strokes in a Dounce homogeniser according to the method of Kidd *et al.* (1983).

RNA was isolated from adult flies as described by Pirrotta *et al.* (1983a). Poly(A)⁺ RNA was prepared by two cycles of binding and elution from a poly(A)-Sephacose column in buffer containing 10 mM Tris pH 7.5, 10 mM EDTA, 0.2% SDS and 0.2 M NaCl. After loading, the column was washed with the same buffer minus salt and eluted with 10 mM Tris pH 7.5, 10 mM EDTA, and 50% deionised formamide.

In situ hybridisation

Salivary glands were squashed on subbed slides in 45% acetic acid, dehydrated through an ethanol series and heat treated 30 min at 65°C in 2 x SSC. The DNA was denatured in 0.2 N NaOH for 2 min, followed by an ethanol series. Hybridisation to a ³H- or ³⁵S-labelled probe took place at 65°C in 1 x Denhardt's solution (Denhardt, 1966), 2 x SSC.

Southern and Northern blot hybridisation

Southern blots were made from agarose gels according to Southern (1975). After baking, the filters were pre-hybridised in 2 x SSC, 10 x Denhardt's solution and 50 μ g/ml denatured salmon sperm DNA. Hybridisation followed under the same conditions. After hybridisation, the filters were washed extensively in 0.1 x SSC, 65°C. For Northern blots, RNA was denatured in 2 M formaldehyde, 50% formamide 5 min at 75°C and loaded on a 1% agarose gel containing 2 M formaldehyde, 0.2 M morpholinopropanesulfonic acid, 50 mM Na acetate. After blotting and baking the nitrocellulose filter was hybridised in 50% formamide, 10 x Denhardt's solution, 100 μ g/ml denatured salmon sperm DNA at 43°C. After hybridisation the filter was washed extensively in 2 x SSC, 65°C and finally in 0.2 x SSC, 65°C.

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