Isolation and characterization of the \textit{zeste} locus of \textit{Drosophila}

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The \textit{zeste} gene of \textit{Drosophila} regulates the expression of certain other genes like \textit{white}, \textit{bithorax} and \textit{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 \textit{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 \textit{zeste} mutants which contain P element sequences inserted in the same region. DNA rearrangements were found inserted in some \textit{zeste} mutants but were not detected in the \textit{z\textsuperscript{1}}, \textit{z\textsuperscript{wp}} or \textit{z\textsuperscript{wp1}} 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 \textit{zeste} mutants but is not visibly affected in \textit{z\textsuperscript{1}} or \textit{z\textsuperscript{wp}} mutants. The region bears no homology to the \textit{white} gene or its vicinity but cross-hybridises to many other genomic sequences in \textit{Drosophila}.

Key words: chromosome pairing\/gene regulation\/transvection/\textit{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 \textit{zeste} (\textit{z}, \textit{X}-1.0) gene of \textit{Drosophila} is a regulatory gene which affects the expression of at least three other loci: \textit{bithorax} (BX-C), \textit{decapentaplegic} (\textit{dpp}) and \textit{white} (\textit{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 \textit{zeste} gene interacts with BX-C, \textit{dpp} and \textit{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 \textit{zeste} gene. Thus, the phenotypic effects of certain combinations of BX-C alleles are alleviated in the presence of \textit{z\textsuperscript{+}} but accentuated by \textit{zeste} mutations (Kauffman et al., 1973). In the \textit{white} locus, the \textit{w\textsuperscript{wp}} mutation (which affects the regulatory region of the gene) can be partially complemented by other \textit{white} alleles such as \textit{w\textsuperscript{a}} (which affects the gene but not its regulatory region) in the presence of \textit{z\textsuperscript{+}} (Babu and Bhat, 1980). The unusual aspect of the \textit{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 \textit{zeste} effect just as mutations in \textit{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 \textit{zeste}. Much of the information on the \textit{zeste} effect comes from the interaction of one particular \textit{zeste} mutation with the \textit{white} locus. This is \textit{z\textsuperscript{1}}, a spontaneously arising allele isolated and analysed by Gans (1953). This mutant has an altered \textit{zeste} gene whose product is still able to interact with the target genes but with the opposite effect: instead of enhancing the expression of \textit{white} or of \textit{dpp}, it depresses it. With respect to BX-C, Kaufman et al. (1973) and Gelbart and Wu (1982) have shown that the \textit{z\textsuperscript{1}} product behaves like the normal product.

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

Most of the mutants in the \textit{zeste} locus are formally equivalent to reduction or loss of function and are referred to as \textit{z\textsuperscript{0}} type. Mutants of the \textit{z\textsuperscript{1}} type are much rarer than the \textit{z\textsuperscript{0}} type, and correspond formally to an altered function.

The genetic results imply: (i) that the \textit{zeste} product has its effect either on the DNA sequence itself or on transcription but not on translation or post-translational processes, (ii) that \textit{zeste} can act also on single copies of the target gene (as shown by the \textit{z\textsuperscript{wp}} mutation) but that the vicinity of more copies of the target gene enhances the effect; (iii) the effect of the \textit{z\textsuperscript{1}} product appears to be antagonistic to that of the \textit{z\textsuperscript{+}} product. Although \textit{z\textsuperscript{1}}/\textit{z\textsuperscript{+}} females have apparently normal expression of \textit{w}, the \textit{z\textsuperscript{1}} effect becomes gradually visible with increasing numbers of tandemly repeated \textit{w} genes (Jack and Judd, 1979). Similarly, \textit{z\textsuperscript{wp}} which behaves as an overproducer of \textit{z\textsuperscript{1}}, is dominant over \textit{z\textsuperscript{+}} in the presence of two paired copies of \textit{w} (Lifschytz and Green, 1984). Analysis of the \textit{white} RA from \textit{z\textsuperscript{1}} male and female adult flies or from \textit{w\textsuperscript{wp}} 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 w10 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" mutants may have residual activity. The molecular details of z" 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)258-11 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-

![Map of the region surrounding the zeste locus](image)
The zeste locus of Drosophila

tation. 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 zmut mutation, strongly sug-

gestng that the 3A3 breakpoint is in or very near the zeste locus. The in situ hybridization 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 EcoRI sites and explains why it was not represented in the microdissection clones, which are limited by the capacity of the vector to EcoRI 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(l)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(l)w258–11, which is very similar to 64c4 and is also deficient for z, has a breakpoint between positions + 1 and + 3. Df(l)s1/11, 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(l)62g62g18 includes z, tko and the giant locus (gt) and has a breakpoint around position + 63 to 64. We

Fig. 2. In situ hybridisation to ln(l) e(bx) chromosomes. The DNA of clone λT17-6 was labelled with [35S]a-thio-nucleotide triphosphates and hybridised to ln(l) e(bx) salivary gland chromosomes. Heavy hybridisation to both ends of the ln(l) e(bx) inversion shows that the clone contains approximately equal amounts of DNA on each side of the breakpoint.

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 BamHI and hybridised with the Bam 4.0 probe. Panel B, C and D show (1) Df(l) w258–11/α-Y DNA, (2) Df(l) 64C4/α–Y DNA, (3) Df(l) w27/α–Y DNA cut with EcoRI and hybridised with the Bam 4.0 probe (panel B), with the Bam 2.4 probe (panel C) with the 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 π2 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 sp1 to reveal zeste mutations. From ~50,000 chromosomes, we found five independent mutants which gave a zeste phenotype in heterozygosis with the y2 z sp1 chromosome. Three of these we found later to be extensive deletions which were homozygous lethal and not further investigated. The remaining two, z6 and zπ2, 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 π2 strain gives no hybridisation to a P probe in this region. When homozygous with the z1 mutation, zπ gives a yellow eye color while homozygous zπ/zπ flies have red eyes. In the zeste terminology, this means that zπ is a z1 or loss of function mutation. However, upon closer inspection, homozygous zπ 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 z1 flies raised at 18°C. The zπ2 mutation when homozygous with z1 gives a yellow-orange eye color indicative of incomplete loss of function. Homozygous zπ2 flies have wild-type eyes.

Revertants of zπ 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 zπ insertion mutant**

The *in situ* hybridisation to zπ 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 zπ 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 π1.4), the fourth was different (type π11.5). 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 π1.4 came from the vicinity of position —70 while clone π11.5 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 π11.5 insertion site to the breakpoint of e(bx) strongly suggested that this and not the π1.4 insertion was responsible for the zπ mutation. We therefore chose the P insertion site in π11.5 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 T71-6. Figure 6 shows that the zπ mutant has an insertion of apparently 0.7 kb at position 0, in agreement with the restriction map of clone π11.5. This mutant also has an insertion near position —70, in agreement with the restriction map of clone π1.4. A revertant of the zπ mutant (zπ*) 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 π11.5 insertion as the one responsible for the mutant phenotype. The second dysgenic mutant, zπ2 also has an insertion at or very near the same site as the π1.5 insertion, but ~1.5 kb larger. Unlike the zπ mutant, zπ2 has no insertion at position —70.
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 HindIII site at position −5 in the Oregon R strain is missing in Canton S, z\textsuperscript{ed}, z\textsuperscript{177h} and in the \pi2 strain and derivatives, while Canton S, z\textsuperscript{1}, z\textsuperscript{ed}, z\textsuperscript{177h}, but not \pi2 have an additional HindIII site at position −8. Similarity, the EcoRI site at position +1.2 is missing in z\textsuperscript{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\textsuperscript{ed} and a fraction of our Oregon R population contain a 2-\textbf{k}b insertion at position +18. In addition, the DNA of the z\textsuperscript{1} mutant contains an insertion of −5 \textbf{k}b 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\textsuperscript{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\textsuperscript{1} called z\textsuperscript{11G3} was isolated by Gans (1953) after X-ray treatment. For these reasons, z\textsuperscript{1} has frequently been supposed to be due to an insertion or similar DNA rearrangement. We hybridised genomic Southern blots of z\textsuperscript{1}, z\textsuperscript{11G3}, z\textsuperscript{p96}, z\textsuperscript{p11} and z\textsuperscript{RNA} with a series of clones spanning 60 \textbf{k}b in the proximal direction and 35 \textbf{k}b 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\textsuperscript{p96} and z\textsuperscript{p11} mutants are two distinct ‘overproducer’ mutants isolated from z\textsuperscript{1} by Lifschytz and Green (1984), while z\textsuperscript{RNA} is a partial revertant of z\textsuperscript{p96}. 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 EcoRI fragments of ~200 \textbf{b}p. Acrylamide gel electrophoresis showed that they are identical in size and that, furthermore, an identical set of fragments is produced by HaeII 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 analysed z\textsuperscript{177}, 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\textsuperscript{1}, behaves like a z\textsuperscript{1} mutant. The genomic Southern blot in Figure 6 shows that z\textsuperscript{177} contains a small deletion of ~300 \textbf{b}p in the 4-\textbf{k}b 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)\textsuperscript{+} RNA from wild-type and several zeste mutants, hybridised with the DNA of \lambda T17-6 (see map in Figure 1). At least three wild-type RNA species hybridise with this probe: a 4.2-\textbf{k}b, a 2.4-\textbf{k}b and a 0.9-\textbf{k}b species. The 2.4-\textbf{k}b species is reduced to 1.8 \textbf{k}b in e(bx), to 2.1 \textbf{k}b in z\textsuperscript{1}, while in z\textsuperscript{177h} it is both shortened to 2.1 \textbf{k}b and is of much lower intensity. No size changes are detectable with z\textsuperscript{1} or z\textsuperscript{ed} RNA although some changes in the relative intensity of...
electrophoresis, blotted onto a nitrocellulose filter and hybridised to the XT 17-6 probe. The mol. wts. were estimated using markers.

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 XT17-6 probe. The mol. wts. were estimated using copia and Adh RNAs as markers.

2 x SSC

0.1 x SSC

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.

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 2.4-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).

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 w115 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 w115 and 62g18 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(l)zwvl (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(l)64c4, and more distal than the breakpoint of Df(l)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 zC17th, zπ and z2. These are all located near position 0 in the map and are all zτ type mutations, if we ignore for the moment the slight reduction in pigmentation and the variegation seen in zC17th and zπ. Both zπ and z2 have sequences of the P transposable element inserted at position 0. The finding that zC17th 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 $z^{27h}$. 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 $z^{p}$ has suffered a deletion in a Bam 4.0-kb fragment containing the insertion site which has removed both P and flanking sequences. Furthermore, the $e(bx)$, $z^{27h}$ and $z^{p}$ 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 $z^{1}$ 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. $z^{1G}$, a partial revertant of $z^{p}$ 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 $z^{p6c}$ 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 $z^{1}$, $z^{p6c}$, 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 $e(bx)$, $z^{p}$ or $z^{27h}$ 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 $z^{p6c}$ DNA, confers the $zeste$ phenotype even in males (Pirrotta and Bozzetti, unpublished experiments) and confirms the single gene interpretation. Mutations like $z^{27h}$, which causes decreased eye pigmentation and variegation, and like $z^{p}$, 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).

$In(1)64B$: 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).

$y^{2}$: carries the $z^{p}$ mutation of Gans (1953).

$z^{27h}$: 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).

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