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The product of the *Drosophila zeste* gene binds to specific DNA sequences in *white* and *Ubx*

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Three different segments of the *zeste* coding sequence were inserted in an expression vector and antibodies were raised against the resulting *zeste*-β galactosidase hybrid proteins. The antibodies were used to analyse the *zeste* protein produced in bacteria from a different expression vector containing the entire *zeste* coding region. The major products made in bacteria as well as the products of *in vitro* translation of *zeste* RNA migrate anomalously upon SDS–acrylamide gel electrophoresis. Specific DNA fragments from the *white* and *Ubx* gene co-immunoprecipitate with *zeste* protein. At least two independent *zeste* binding sites are found in a 250-bp interval of the *white* regulatory region that contains also the sites of w^{sp} mutations, which are known to be deficient in *zeste* interaction.

Key words: transvection/enhancers/*white* and *Ubx* genes

Introduction

Two unusual genetic phenomena in *Drosophila* suggest that gene expression and its regulation may be affected by the configuration of chromosomes in the nucleus. The two phenomena, transvection and the *zeste–white* interaction, involve the product of the *zeste* gene and the state of pairing of the chromosomes bearing the target genes. Transvection (Lewis, 1954; Kaufman *et al.*, 1973; Gelbart and Wu, 1982) may be understood as the effect of the regulatory region of one gene copy on the expression of another copy of the gene present on the homologous chromosome. A particularly illuminating example is the transvection effect observed in heterozygous flies with the genotype *Cbx Ubx*/+ +, where *Ubx* is a mutation in the coding region of the *Ubx* locus while *Cbx* is a regulatory mutation of *Ubx* that has been shown to result in its inappropriate expression (White and Akam, 1985). Transvection effects, observed when the doubly mutant and the fully normal gene copies are somatically paired, cause the inappropriate expression of the wild-type *Ubx* gene under the influence of the *Cbx* regulatory mutation in the mutant *Ubx* gene (Lewis, 1954; Gelbart and Wu, 1982). Transvection effects require the physical juxtaposition of the two gene copies by somatic pairing of the homologous chromosomes as well as the presence of an active *zeste* gene. At least three loci display *zeste*-dependent transvection effects: the *Ubx* unit of the *bithorax* complex, the *decapentaplegic* locus (*dpp*) and the *white* locus. Other loci have been reported to display similar effects (Ashburner, 1970; Korge, 1981; Kornher and Brutlag, 1986) but their dependence on *zeste* is not known.

The second pairing-dependent regulatory effect is that exerted by the z^1 mutant product on the expression of the *white* gene (Gans, 1953). In the presence of this particular allele of *zeste*, the expression of *white* in the eye is drastically reduced and the level of *white* RNA found in the head of the fly decreases by more than 90% (Bingham and Zachar, 1985). This effect, like transvection, is pairing dependent. It requires the presence of two copies of the *white* gene in close physical proximity, achieved either by homologous chromosome pairing or by a tandem duplication of *white*.

The existence of transvection effects is revealed in the presence of certain combinations of mutations in the two copies of the target gene. We do not know what role the *zeste* product plays normally in gene regulation or how it interacts with single genes. The effect of pairing, however, strongly suggests that *zeste* acts at the level of the gene, either by controlling transcription or by affecting some post-transcriptional process closely associated with it. Genetic and molecular analysis of the *white* locus have shown that the *zeste* effect requires sequences in the *white* regulatory region located in a 116-bp interval about 1200 bp upstream of the transcriptional start site (Pirrotta *et al.*, 1985; Davison *et al.*, 1985). Moreover these sequences may be brought closer or further away or inverted with respect to the promoter without affecting their ability to interact with *zeste*. These results indicate that the target of *zeste*, whether direct or indirect, is a DNA sequence in the *white* regulatory region which behaves in an enhancer-like fashion.

Several possible hypotheses could be entertained about the role of *zeste* in chromosome interactions and gene expression. One possibility is that it is a *trans*-acting factor that activates an enhancer element common to *Ubx*, *dpp* and *white*. Transvection phenomena then suggest that enhancer elements on one DNA molecule could control promoters on a second DNA molecule, provided the two are held in place in a suitable spatial configuration.

Another possible interpretation is that *zeste* is not itself an enhancer-activating factor but contributes to anchor genes to some nuclear structure where transcriptionally active complexes are assembled. None of the current hypotheses is fully satisfactory in explaining all the observations and, in particular, the different effects of *zeste* mutants on the behavior of different loci (see Pirrotta *et al.*, 1987).

In this paper we report the first molecular studies of the *zeste* gene product and its function and we demonstrate that the *zeste* protein binds directly to specific DNA sequences of the *white* and *Ubx* regulatory region.

Results

Zeste antibodies

To study the *zeste* gene product we first undertook the preparation of antibodies directed against parts of the *zeste* protein. The nucleotide sequence of the *zeste* gene predicts that the central part of the protein encoded will have several extensive runs of Gln, of Ala and of alternating Gln Ala (Pirrotta *et al.*, 1987). Such sequences have been found in several other *Drosophila* coding regions where they have been variously referred to as *opa* sequences (Wharton *et al.*, 1985) or M repeats (Schneuwly

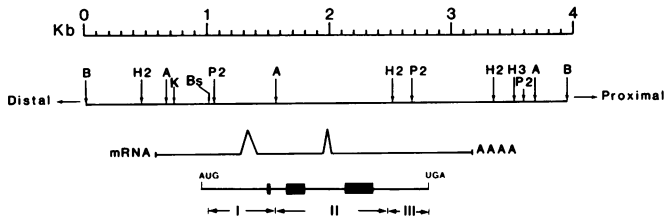

Fig. 1. Map of the *zeste* gene. The restriction sites are B = *Bam*HI, H2 = *Hin*dII, A = *Ava*I, K = *Kpn*I, Bs = *Bst*EII, P2 = *Pvu*II, H3 = *Hin*dIII. The position of the transcribed region, with two introns, is shown in the third line. The bottom line shows the positions of the translation start and termination codons while the black boxes symbolize the Gln or Gln Ala repetitive regions. The open reading frame fragments used to make fusion proteins with which to raise antibodies are marked I, II and III.

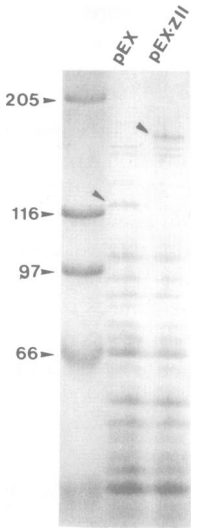

Fig. 2. Gel electrophoresis of trihybrid protein. Extracts from bacteria containing vector alone (pEX) and vector with the *zeste* open reading frame fragment II (pEX-zII) were run on a 7.5% SDS–acrylamide gel along side mol. wt markers. The gel was stained with Coomassie Brilliant Blue. The *cro*-β galactosidase and the trihybrid protein are indicated by the arrowheads. Mol. wts are in kd.

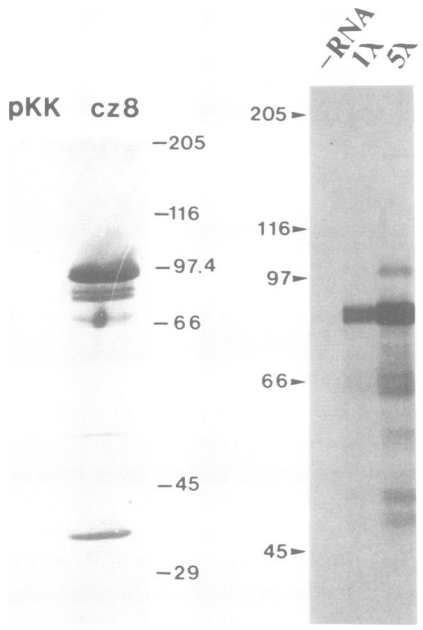

Fig. 3. *Zeste* protein made in bacteria or by *in vitro* translation. The **left panel** shows a Western blot of extracts from bacteria containing the pKK223-3 vector alone (pKK) or vector bearing the *zeste* cDNA (cz8). The blot was reacted with antiserum II and stained with peroxidase. The top band, with mobility corresponding to 97 kd is variable in intensity and was much weaker in some experiments. The **right panel** shows an autoradiograph of the products of *in vitro* translation of *zeste* RNA. The three lanes show the products in the absence of added RNA (5 μl of translation reaction) or with 1 μg *zeste* RNA made by *in vitro* transcription (1 and 5 μl of translation reaction). Mol. wts are in kd.

et al., 1986) and are frequent in *Drosophila* poly(A)$^+$ RNA. Consequently, a number of other *Drosophila* proteins might share antigenic epitopes with *zeste*. We therefore subdivided a full length *zeste* cDNA clone into three intervals, shown schematically in Figure 1. Of these, the 3′-most fragment does not code for any Gln Ala runs, the 5′ fragment contains only a small run of six Gln, while most of the repetitive sequence is confined to the middle fragment. We cloned these fragments, in the appropriate reading frame, in the pEX expression vectors of Stanley and Luzio (1984). These vectors are designed to express a trihybrid protein composed of λ *cro*, β-galactosidase and the peptide encoded by the inserted open reading frame sequence, under the control of the λ P_R promoter. The clones we obtained, after heat induction to inactivate the λ *c*I ts 857 repressor, produce a hybrid protein of the expected mol. wt. Figure 2 shows the fusion product with the middle *zeste* fragment as an example.

We excised the trihybrid protein band from a preparative acrylamide gel, homogenized it and injected the suspended material into mice to raise antibodies.

Expression of zeste in bacteria

The three antisera prepared from immunized mice recognize β-galactosidase, but fail to detect cross-reacting proteins in crude extracts made from pupae or adult flies (not shown). To study the properties of the *zeste* protein, we therefore constructed an expression clone to produce the protein in *Escherichia coli*. The *zeste* coding sequence was taken from a full-length cDNA clone, cut at the *Kpn*I site to trim away part of the 5′ untranslated leader and inserted in the bacterial expression vector pKK223-3. In this vector [one of a family described by Brosius (1984)] the inserted gene is transcribed under the control of the inducible *tac* promoter. Extracts prepared from induced cultures carrying the expression plasmid were analysed by SDS gel electrophoresis and Western blotting. Figure 3 shows the results of a typical experiment. Antiserum raised against the central part of the *zeste* protein (region II in Figure 3) does not cross-react with a control extract made from bacteria containing the pKK223 vector with no insert but reveals multiple bands in extracts from bacteria carrying the pKK-z recombinant plasmid. Antisera I, II and III gave very similar results, differing only in the minor bands visible in the Western blot. The pattern of bands revealed by the antibodies is unexpected. The low mol. wt bands in Figure 3 could be readily explained as degradation products, not surprising in view of the unusual amino acid structure of the *zeste* protein. However, the top bands, which are also the most intense although their relative strengths vary in different preparations, migrate with an apparent mol. wt of 89–97 kd, while the mol. wt predicted from the *zeste* DNA sequence is 61 000. Since the DNA fragment inserted in the pKK223 vector contains terminator codons in all three frames in the region immediately following the *zeste* coding region, and since the vector contains strong transcriptional terminators after the cloning site, the large peptides could not

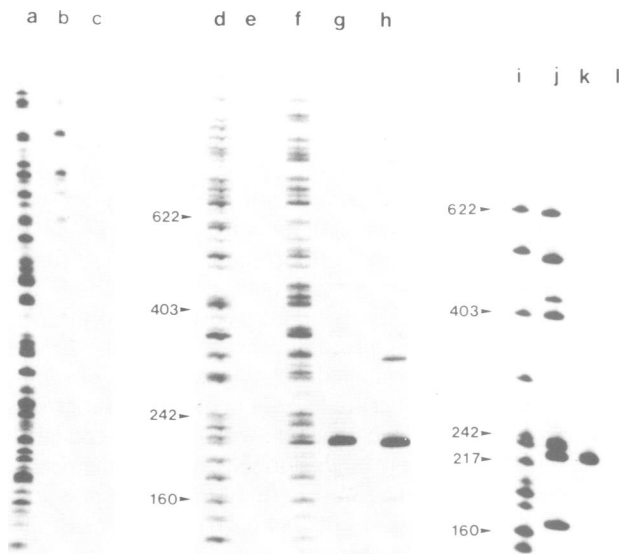

Fig. 4. Specificity of binding of *zeste* protein to DNA. DNAs were cut with *Dde*I, end labelled, incubated with bacterial extracts and the fragments immunoprecipitated were then analysed on acrylamide gels. The **left panel** shows non-specific binding to λDNA: **(a)** total λDNA fragments, **(b)** λ DNA fragments immunoprecipitated in the presence of *zeste* extracts with no competing calf thymus DNA, **(c)** plus 4 μg calf thymus DNA. All other binding reactions always included calf thymus DNA. The **middle panel** shows the binding to the λEMBL4 w11 clone: **(d)** total λEMBL4 vector DNA, **(e)** λEMBL4 DNA binding with 20 μg *zeste* extract protein, **(f)** total λ w11 DNA, **(g)** λ w11 DNA binding with 20 μg *zeste* extract protein, **(h)** λ w11 DNA with 60 μg *zeste* extract protein. The **right panel** shows binding to the pUC-Dde 224 subclone: **(i)** marker DNA, **(j)** total pUC-Dde 224 DNA, **(k)** pUC-Dde 224 DNA binding with 15 μg *zeste* extract proteins, **(l)** pUC-Dde 224 DNA binding with 15 μg protein extract from bacteria containing the pKK223-3 vector with no *zeste* insert. The material run in **lanes (a), (d), (f)** and **(j)** represents only a fraction of the total input DNA in the binding reactions.

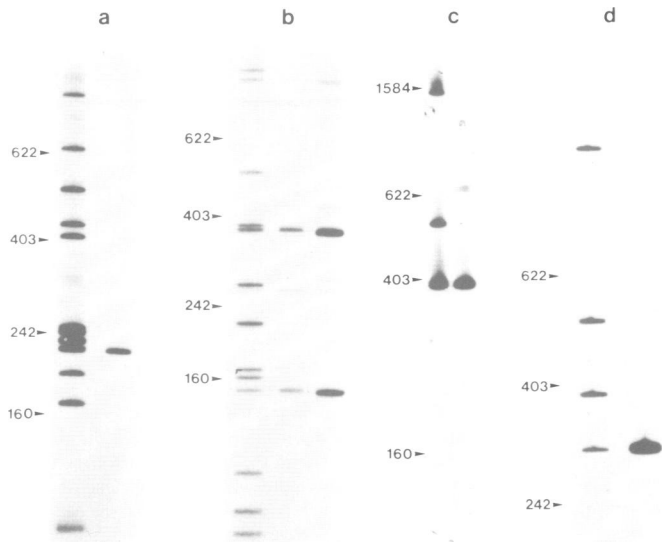

Fig. 5. Mapping of the *zeste* binding sites in the *white* gene: **(a)** binding to clone pUCHH cut with *Dde*I; **(b)** binding to clone pUCHH cut with *Hin*f with standard amounts and double standard amounts of extract; **(c)** binding to pUC Dde 224 cut with *Hin*fI; **(d)** binding to pUC DdBg 130 cut with *Hin*fI.

plausibly be attributed to translational read-through. We considered three alternative explanations: (i) the protein becomes covalently modified in bacteria; (ii) the protein forms associations that are resistant to denaturation; and (iii) the protein simply migrates anomalously either because of unusual shape or through failure to bind SDS molecules in proportion to its size. We found no change in electrophoretic migration after boiling in SDS in the presence or absence of 10 mM β-mercaptoethanol, suggesting that disulphide bridges are not involved. Non-covalent oligomerization that is resistant to boiling has been observed in certain proteins (Gazin *et al.*, 1986). It is surely very unusual but cannot at present be ruled out.

In vitro translation of zeste RNA

To clarify the situation, we recloned the cDNA fragment in the pSP65 vector and transcribed it *in vitro* using SP6 RNA polymerase. The RNA, made from a template linearized by cleavage in the polylinker, was then translated *in vitro* with a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Figure 3 shows that the products of *in vitro* translation are very similar to those detected by Western blots of the bacterial extracts. In particular, the major products form a doublet migrating with an apparent mol. wt of 89−94 kd. This corresponds well to the doublet of similar size seen in the Western blot of the bacterial extracts. However, the major band in the Western blot, with an apparent mol. wt of 97 kd, is represented only weakly in the *in vitro* translated product. We do not know whether the many faster migrating bands are due to degradation, premature termination or internal translation starts. However, precipitation with the *zeste* antisera gives a qualitatively similar band pattern.

Zeste protein binds to DNA

The previous results provide convincing evidence that the expression clones produce proteins encoded by the *zeste* gene and that our antisera cross-react specifically with the same proteins. We next investigated the ability of these proteins to interact with end-labelled DNA fragments from the *Drosophila white* gene. Bacterial extracts were prepared in 4 M urea which was then removed by stepwise dialysis to facilitate renaturation of the proteins. Because the extracts contain many bacterial DNA binding proteins, we used immunoprecipitation with the *zeste* antisera to select only those complexes including *zeste* protein. The DNA fragments co-precipitated by the antibody were then analysed on an acrylamide gel.

Figure 4 shows that although several fragments of λDNA digested with *Dde*I are still co-precipitated by this procedure, the non-specific binding can be competed away by adding sufficient amounts of unlabelled calf thymus DNA. Under these conditions, a single *Dde* fragment of 224 bp is co-precipitated from a digest of λEMBL4 w11, a genomic clone containing 17 kb of *Drosophila* DNA including the entire *white* gene and ~9 kb of 5′ flanking sequences (Pirrotta *et al.*, 1983). Increasing amounts of *zeste* extracts in the binding reaction reveal the existence of secondary binding sites, which we estimate to be at least ten times weaker under these conditions.

Restriction mapping and subcloned fragments show that the major *zeste* binding site is located in the 5′ flanking region, ~1200 bp upstream of the transcription start site, in agreement with the genetic data. From the DNA sequence of the *zeste* gene determined by O'Hare *et al.* (1984) we find that the *Dde* 224-bp fragment spans the sites of the w^{sp1} insertion and the w^{sp2} deletion, two mutations that fail to interact with *zeste*. We subcloned this fragment in the pUC8 plasmid and confirmed its interaction with *zeste*. Figure 4 shows that this fragment is selectively immunoprecipitated from a *Dde*I digest of pUC8-Dde 224 in the presence of *zeste* extracts but not with extracts from bacteria containing the pKK223 vector without *zeste* gene.

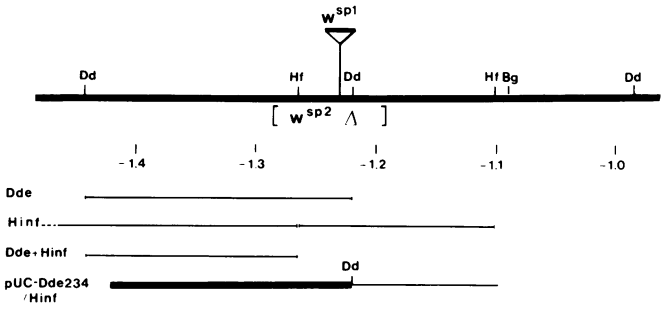

Fig. 6. Map of the *zeste* binding region of *white* and summary of binding results. Restriction sites are indicated by Dd = *Dde*I, Hf = *Hin*fI, Bg = *Bgl*II. The site of the 8.7 kb w^{sp1} insertion and the extent of the 116 bp w^{sp2} deletion are also indicated. Below the map are summarized the results of the binding experiments. The heavy bar in the last line symbolizes vector DNA. The scale is in kb from the transcription start site determined by S1 mapping (Steller and Pirrotta, 1985) which corresponds to position +3687 in the sequence of O'Hare *et al.* (1984). Note that some polymorphisms exist between our sequence and theirs resulting, for example, in the absence of a *Hin*fI site at −1487 (+5174 of O'Hare *et al.*).

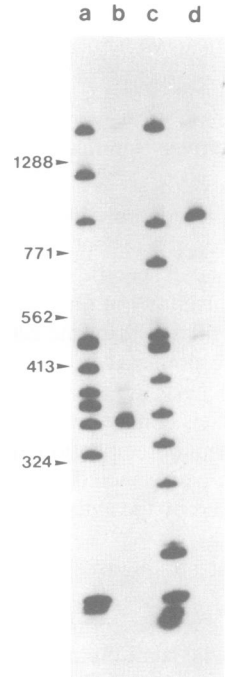

Fig. 7. *Zeste* binding to *Ubx* DNA: **(a)** *Dde*I digest of clone p3102B containing 3.5 kb with the transcription start site and 5′ flanking sequences of the *Ubx* gene; **(b)** binding fragments from the DNA in **(a)**; **(c)** p3102 cut with *Hin*fI; **(d)** binding fragments from the DNA in **(c)**.

Zeste binds to at least two independent sites

Figure 5 shows binding reactions using pUCHH, a subclone containing the *white* region from the *Bgl*II site at position −1088 to the *Hin*dII site at position −1859, measured from the transcription start site (Steller and Pirrotta, 1985). Although only the *Dde* 224 fragment is bound from a *Dde* digest, two fragments are immunoprecipitated from a *Hin*f digest under the same conditions. The *white* DNA sequence, as well as restriction mapping data, shows that these two *Hin*f fragments, of 344 and 163 bp, are adjacent and both overlap the *Dde* 224 fragment. This finding indicates that there are at least two sites able to bind independently to *zeste*.

The simplest interpretation of these results is that both binding sites are contained in the *Dde* 224 fragment and are separated by the *Hin*f site at position −1262. However, when the pUC-Dde 224 subclone is cut with *Hin*f, only one fragment, corresponding to the upstream part of the clone, appears to bind to *zeste*. A possible explanation for this discrepancy is that one binding site is disrupted by cleavage with *Dde* at position −1218. We investigated the binding activity of the adjacent *Dde*I−*Bgl*II fragment, representing the 130 bp sequence from −1088 to −1218. This fragment was made blunt-ended by filling in the ends with Klenow DNA polymerase and ligated to the *Hin*dII site of pUC8. Since this procedure reconstitutes the *Dde* site, the pUC-DdBg130 clone contains three nucleotides of *white* sequence at the *Dde* end that are lacking in the fragment produced by *Dde* digestion. This subclone, cut with *Hin*f and end labelled gives specific and efficient binding to the expected fragment consisting now of most of the *Dde*−*Bgl*II 130 sequence plus 205 bp of plasmid DNA. These results, summarized schematically in Figure 6, indicate that there is a *zeste* binding site in the *Dde*I−*Bgl*II 130 fragment but that efficient binding requires the presence of the full double-stranded sequence up to and including the *Dde* recognition site at position −1218.

Zeste binds to Ubx regulatory sequences

Since the *Ubx* gene exhibits transvection effects that depend on the *zeste* gene, the results obtained with *white* suggested that *zeste* would bind to *Ubx* DNA. We screened genomic clones spanning 70 kb and including the *bxd* region and the 5′ end of the *Ubx* transcription unit [positions +31 to −39 in the *Ubx* map of Bender *et al.* (1983)]. We found a single DNA fragment that bound to *zeste*: an *Eco*RI−*Hin*dIII fragment that contains the transcription initiation site of *Ubx*. Figure 7 shows the binding of a plasmid subclone p3102B that contains 3.5 kb including ~3 kb of 5′ flanking sequences. Both *Dde*I and *Hin*fI digestions reveal a single fragment that binds to *zeste* extracts. The exact location of these fragments was determined by direct sequencing of the *Dde* fragment (K.L.Chow and V.Pirrotta, unpublished) and comparison with the *Ubx* sequence (P.Harte and D.Hogness, unpublished) and corresponds to a segment spanning the *Ubx* transcription start site.

Discussion

The genetic data and the results of germ line transformation with transposons containing altered *white* genes intimated that the product of the *zeste* gene interacts with DNA. From the genetic viewpoint, however, there are strong indications that *zeste* must interact with other gene products: there exist suppressor of *zeste* and enhancer of *zeste* loci (Green, 1967; Persson, 1976; Wu, 1984); *zeste* mutations do not have the same effects on different target genes (Gelbart and Wu, 1982). The germline transformation experiments also show that the genomic environment has an important effect on the interaction of *zeste* with *white*: the same $white^+$ transposon in different genomic sites may show different levels of *zeste* effects ranging from undetectable to extreme (Hazelrigg *et al.*, 1984; Pirrotta *et al.*, 1985). It was therefore possible that *zeste* intervened at a later stage in the interaction of a DNA target with other nuclear components. The results presented in this paper show instead that the product of the *zeste* gene binds directly to specific DNA sequences in the absence of other *Drosophila* proteins. The involvement of other factors or nuclear components is still strongly implied but it must occur independently or subsequently.

The DNA region of the *white* gene to which the *zeste* protein

binds coincides closely with that defined by two mutations that cause failure to interact with *zeste*. One of these is w^{sp2}, a 116-bp deletion whose position is indicated in Figure 6. This deletion clearly eliminates the *zeste* binding site associated with the *Dde*I recognition sequence at position −1218. We do not yet know if the additional binding site or sites are also missing in this mutant. If so, they must be located in the 30-bp interval between −1292 and −1262. It is possible that they are still present, if functional *zeste* interaction requires the occupation of multiple sites. The w^{sp1} mutation suggests that the integrity of the multiple binding sites might be important. In this mutant, a B104 transposable element of 8.7 kb has inserted at position −1233 (Zachar and Bingham, 1982; O'Hare *et al.*, 1984). This could not affect the *zeste* binding site associated with the *Dde* sequence at −1218 nor the binding site located upstream of the *Hin*f site at −1262 but it would interpose between them 8.7 kb of foreign DNA. We conclude therefore that either the physical proximity of the two sites is essential for the bound *zeste* protein to carry out its function or that some sequence in the B104 element actively interferes with this function, possibly by competing with the *white* promoter for the attentions of the bound *zeste* product or even by steric hindrance due to proteins bound to the B104 ends.

The *zeste* dependence of transvection at the *Ubx* locus implies that *zeste* protein also interacts with the *Ubx* regulatory region. In contrast to *white*, however, there are no available genetic data for *Ubx* to help pinpoint the site of interaction. On the contrary, mutations that can be interpreted as having regulatory effects are found both in the 40 kb preceding the transcription start and in the 70-kb transcription unit (Hogness *et al.*, 1985). It was reassuring therefore to find a single *zeste* binding region in the 5′ flanking region, in the immediate vicinity of the transcriptional start site. Although we found no other binding region in the interval we tested, which includes 50 kb of 5′ flanking sequence and 10 kb of transcribed sequence, we have not yet screened the entire *Ubx* locus.

Work is in progress to determine the nucleotide sequence of the *zeste* binding sites and the frequency of such sites in the regulatory regions of *Drosophila* genes. More interestingly perhaps, we now have the tools to examine the localization of *zeste* protein in nuclei and its interactions with other components.

Materials and methods

Preparation of antigen and antisera

Expression clones were constructed by inserting the open reading frame fragments from *zeste* cDNA clone shown in Figure 1 into the appropriate pEX vector and introducing the resulting plasmid in the NF1 host bacteria (Stanley and Luzio, 1984). To prepare antigen, 100 ml cultures were grown at 30°C to OD_{600}l and then shifted to 43°C for 2 h. The bacteria were pelleted, resuspended in gel sample buffer (100 mM Tris−HCl pH 8.8, 4% SS, 1 mM EDTA, 10 mM DTT, 15% sucrose and bromophenol blue) and boiled for 5 min. The material was then centrifuged and the supernatant was applied to a 2-mm-thick 7.5% acrylamide gel containig 1% SDS. The gels were stained for 1 h with Coomassie Brilliant Blue and the band corresponding to the trihybrid protein was excised with a scalpel. The acrylamide slice was chopped into small pieces, suspended in 2.5 ml Complete Freund's Adjuvant plus 2.5 ml water and homogenized by vigorous and repeated passage through a syringe needle. Aliquots of the suspension (about 1/10) were injected into each mouse intraperitoneally. Booster injections were administered every 3 weeks and the mice were bled 4−8 days following the boosters. Antibodies to region I, II and III gave qualitatively similar results in the different experiments.

Construction of expression clones and preparation of extracts

To construct a clone that would express *zeste* protein we used a full length cDNA clone (Pirrotta *et al.*, 1987). Since the cDNA contains ~400 nucleotides of untranslated sequence, including several AUG codons, we trimmed the leader by cutting a pUC8 clone containing the cDNA with *Kpn*I and digested briefly with *Bal*31. The trimmed cDNA, still containing ~200 nucleotides of leader sequence, was excised from the plasmid and ligated in the *Sma*I site of pKK223-3. This expression vector utilizes the inducible *tac* promoter and contains strong transcriptional terminators. It was constructed by J.Brosius and is described in the *Pharmacia Molecular Biologicals* catalogue. The construction was introduced into JM109 bacteria which carry a $lacI^Q$ repressor gene to repress the *tac* promoter. To prepare extracts cells were grown in L broth containing 50 μg/ml ampicillin to A_{600} = 0.7. IPTG was added to a final concentration of 10^{-3} M and growth continued for 2 h (Amann *et al.*, 1983). Extracts were made essentially according to Desplan *et al.* (1985). The bacteria were resuspended in 0.5% culture volume of buffer containing 25% sucrose, 0.2 mM EDTA, 40 mM Tris pH 7.5 and 1 mM dithiothreitol. Lysozyme was added at 0.4 mg/ml and, after 1 h at 0°C, urea to 4 M and incubation was continued 1 h. The extract was sonicated to fragment bacterial DNA and then centrifuged at 20 000 r.p.m. for 1 h. The supernatant was dialysed first against buffer containing 10 mM Tris pH 7.5, 25 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 1 mM DTT, 10% glycerol, 1 mM phenylmethyl sulphonyl fluoride, 0.1 mM benzamidine and 2 M urea, then against the same buffer minus urea.

Western blots

Samples were applied to a 10% acrylamide−SDS gel (Dreyfuss *et al.*, 1984). After electrophoresis the gel was electroblotted onto a nitrocellulose filter using a Sartorius semi-dry electroblotter. The filter was blocked overnight in a solution containing 3% milk solids in TBS buffer (10 mM Tris pH 7.4, 0.9% NaCl). After washing the filter 2 × 10 min in the same solution, the antiserum was added at a 1:200 dilution and incubation continued 2 h at room temperature. After washing 2 × 10 min, biotinylated anti-mouse IgG (Vectastain) was added at a 1:200 dilution and incubation continued 1 h. The filter was washed 2 × 10 min and avidin-conjugated peroxidase (Vectastain) was added for 1 h followed by washing in TBS without milk solids 2 × 10 min and developed for 5 min in 0.05 M Tris pH 7.2, 0.03% hydrogen peroxide, 0.5 μg/ml diaminobenzidine and 0.04% $NiCl_2$.

In vitro transcription and translation

DNA from a pSP65 construct containing the cDNA clone was linearized with *Hin*dIII and transcribed using SP6 RNA polymerase in the presence of 0.5 mM GpppG (Promega Biotec) as described by the manufacturer, to obtain capped *zeste* mRNA. The RNA was precipitated from the transcription mixture using 3 M LiCl and reprecipitated with ethanol. Translation was carried out using a rabbit reticulocyte extract (Promega Biotec) in the presence of [^{35}S]methionine.

DNA binding reactions

We followed, with modifications, the procedure of Desplan *et al.* (1985). The DNA to be tested for binding was cut with a suitable restriction enzyme and end labelled using the Klenow fragment of DNA polymerase. The labelled DNA (30 ng) was incubated with bacterial extracts (15−20 μg protein) prepared as described above in the presence of 2.25 μg calf thymus DNA in 25 μl binding buffer (20 mM Tris pH 7.5, 150 mM NaCl, 0.25 mM EDTA, 1 mM DTT, 10% glycerol). After 30 min at 0°C, 1 μl antiserum was added and incubation continued 30 min. Protein A−Sepharose (Pharmacia) was then added (25 μl of 0.2 g/ml) and after 30 min more the mixture was diluted with 300 μl binding buffer, centrifuged briefly, the pellet was washed twice with 350 μl binding buffer and phenol extracted. The DNA was ethanol precipitated and analysed on acrylamide gels. The efficiency of the binding and immunoprecipitation reactions was generally 5−10%. That is, 5−10% of the input radioactivity in the binding fragment was recovered.

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