# Identification of Mutations in Three Genes That Interact With *zeste* in the Control of *white* Gene Expression in *Drosophila melanogaster*

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ABSTRACT

Three previously described genes, *enhancer of yellow, 1, 2* and *3*, are shown to cooperate with the *zeste* gene in the control of *white* gene expression. The mutations $e(y)1^{u1}$, $e(y)3^{u1}$, and to a lesser extent $e(y)2^{u1}$, enhance the effect of the *zeste* null allele $z^{v77h}$. Different combinations of $e(y)1^{u1}$, $e(y)2^{u1}$ and $e(y)3^{u1}$ mutations with several other *z* alleles also enhance the *white* mutant phenotype, but only to levels characteristic of *white* alleles containing a deletion of the upstream eye enhancer. Loss of zeste protein binding sites from the *white* locus does not eliminate the effect of $e(y)1^{u1}$ and $e(y)3^{u1}$ mutations, suggesting that the products of these genes interact with some other nucleotide sequences. Combinations of either $e(y)1^{u1}$ or $e(y)2^{u1}$ mutations with $e(y)3^{u1}$ are lethal. The products of these three genes may represent, together with zeste, a group of proteins involved in the organization of long-distance interactions between DNA sequences.

The regulatory elements that modulate the expression of eukaryotic genes may be positioned tens of kilobases distant from the promoter they control. Accumulating evidence supports the idea that enhancer-bound factors interact with the promoter region by looping out the intervening DNA (PTASHNE 1988; MULLER and SCHAFFNER 1990). A special class of proteins might be involved in organizing the interaction between genomic DNA sequences located large distances apart (PIRROTTA 1990; WU and GOLDBERG 1989). Such proteins may be very important for the structural organization of giant DNA loops, or domains (GEORGIEV *et al.* 1991), in a manner favorable for transcription.

One such protein is the product of the *zeste* gene in *Drosophila melanogaster*, which has been shown to control long distance interactions in chromosomes (PIRROTTA 1990). This gene encodes a protein able to specifically bind to transcriptional enhancers (BENSON and PIRROTTA 1987) and also to the immediate vicinity of transcription start sites (BENSON and PIRROTTA 1988). The zeste protein can also form multimeric complexes connecting distantly located regions of DNA to one another (BICKEL and PIRROTTA 1990). Mutations in the zeste locus influence the expression of several genes, in particular *white*.

The *white* gene has an eye enhancer between positions −1084 and −1856 from the cap site (LEVIS *et al.* 1985; PIRROTTA *et al.* 1985) which consists of apparently redundant sub-elements and contains five binding sites for the zeste protein (BENSON and PIRROTTA 1988). Two other zeste protein binding sites are located in the promoter area. Some basic level of transcription of the *white* gene (yellow eyes) is supported by the proximal promoter element alone. In the presence of the upstream enhancer, transcription reaches normal levels (LEVIS *et al.* 1985; PIRROTTA *et al.* 1985). Based on these results, it is possible to conclude that the zeste protein is involved in the formation of contacts between promoter and enhancer.

However, a complete lack of *zeste* function only decreases eye pigmentation to a dull brown color. Thus, *zeste* does not play an essential role in normal *white* expression (GOLDBERG *et al.* 1989). Point mutations changing the specificity of the zeste protein have a much stronger effect on *white* gene transcription. The classical *zeste-white* interaction is a severe repression of the *white* gene in the presence of the $z^1$ mutation (GANS 1953; BINGHAM and ZACHAR 1985). In the presence of a single copy of *white*, the $z^1$ mutation has no detectable effect on wild-type eye pigmentation, while the pigmentation is reduced to a yellow color with two paired copies of *white* (JACK and JUDD 1979). If the *white* locus is translocated in females, thus preventing interaction between homologous elements, the phenotype induced by $z^1$ is suppressed (GELBART 1971). These observations indicate an involvement of the zeste protein in transvection, *i.e.*, the interaction between regulatory elements located in homologous chromosomes. This interaction has been well documented in *Ultrabithorax, decapentaplegic* and *yellow* (LEVIS 1954; GELBART and WU 1982; GEYER *et al.* 1990).

The zeste protein may either participate in the formation of contacts between enhancers and promoters, or disturb this interaction by the formation of alternative contacts with other zeste protein binding sites (WU and GOLDBERG 1989). The mutations in the $z^1$ and $z^{Op6}$ alleles affect the C-terminal half of the zeste protein, near the

domain responsible for self-aggregation, and may represent sites of interaction with the other proteins (BICKEL and PIRROTTA 1990). These mutations may change contacts formed by zeste proteins. A similar situation has been described for the AraC protein which inhibits the transcription from the araBAD promoter by DNA looping between AraC protein bound at two sites near the promoter (LOBELL and SCHLEIF 1990).

A direct deletion analysis of the *white* gene shows that zeste protein binding sites in the enhancer and promoter regions do not play an important role in transcription activation (QIAN *et al.* 1992). However, a so called anchor site was detected in the *white* promoter region (from −17 to −113). Removal of this sequence completely blocks the effect of the enhancer. This element may be responsible for functional loop formation and would mask the role of zeste protein in the organization of enhancer-promoter interactions (QIAN *et al.* 1992). One can suggest that some other proteins compensate for the lack of zeste protein product. For example, they may be involved in linking enhancers to the above mentioned anchor site.

The aim of the work described here was to find genes that genetically interact with the *zeste* null allele $z^{v77h}$. In a previous study (GEORGIEV and GERASIMOVA 1989), we described six new genes designated as *enhancers of yellow*, that mutant-enhanced many different mutations in the *yellow* locus. In particular, they strongly enhance the $y^2$ allele induced by mdg4 (*gypsy*) insertion. Herein, mutations in the *e(y)* loci were analyzed for their ability to function as enhancers of $z^{v77h}$. It was found that three genes, *e(y)1*, *e(y)2* and *e(y)3*, interact with zeste in the control of *white* gene expression. Combinations of either the $e(y)1^{u1}$ or $e(y)2^{u1}$ mutations with $e(y)3^{u1}$ result in lethality. One can suggest that these three genes belong to the same family, have similar functions and can compensate for one another. They may represent, together with *zeste*, a group of genes involved in the organization of long-distance DNA interactions.

## MATERIALS AND METHODS

**Fly culture:** Flies were cultured at 25 ± 1° or 18 ± 1° on standard Drosophila wheatmeal, yeast, sugar and agar medium. In general, three females were mated with three to five males in vials and brooded every other day.

**Description of mutations:** Balancer chromosomes and most mutations are described in LINDSLEY and ZIMM (1992). $Su(z)2^1$, $Su(z)2^4$, $Su(z)2^5$, $Psc^1$, $Scm^{D1}$, *Su(z)302*, *Su(z)301*, $E(z)^1$ were provided by C.-T. WU.

The following *zeste* mutations have been used in this work. $z^{v77h}$ is a deletion of the leader sequence of the *zeste* gene, resulting in the complete disappearance of the zeste protein product (PIRROTTA *et al.* 1987); $z^1$ (GANS 1953) and $z^{Op6}$ (LIFSCHYTZ and GREEN 1984) are point mutations that change the behavior of the zeste protein (PIRROTTA *et al.* 1987). All these mutations were provided by V. G. CORCES. $z^{Op6R1}$ is a partial reversion of $z^{Op6}$ obtained in our group (P. G. GEORGIEV, unpublished). The $z^{Op6R1}$ mutation is characterized by the absence of dominant effects on the $w^+$ allele. $z^{Op6R1}$ males have darker eyes than $z^{Op6}$ males.

The following *white* mutations were used. The $w^a$ allele is due to a *copia* insertion in the second intron, and the $w^{bf}$ allele to a roo insertion in the third intron of the *white* locus (ZACHAR and BINGHAM 1982). $w^{sp1}$ was induced by a roo (B104) insertion at position −1170 (from the cap site), where the upstream enhancer of *white* is located. The $w^{sp2}$ and $w^{sp4}$ mutations are deletions of the regions from −1231 to −1115 and −2200 to −1029, respectively (DAVISON *et al.* 1985). All these mutations were provided by the Mid-America Stock Center at Bowling Green.

The mutations $e(y)1^{u1}$, $e(y)2^{u1}$ and $e(y)3^{u1}$ were described earlier (GEORGIEV and GERASIMOVA 1989; GEORGIEV *et al.* 1990). All *enhancers of yellow* are located in the *X* chromosome: *e(y)1*, 1–57.9 (16B); *e(y)2*, 1–36.2 (10C); *e(y)3*, 1–62.2 (18CD).

**Construction of combinations of *enhancers of yellow* and other mutations:** Combinations of $z^{v77h}$ (or any other *z* allele) and any of the $e(y)1^{u1}$, $e(y)2^{u1}$ or $e(y)3^{u1}$ alleles were carried out according to the following scheme:

$F_0$ ♀ $e(y)*/FM4$ × ♂ $y^{2\ v77h}m\ f\ Bx^2/Y$;
$F1$ ♀ $e(y)*/y^2\ z^{v77h}m\ f\ Bx^2$ × ♂ $FM4$;

$F_2$ selection of ♂ $y^2\ zv^{77h}\ e(y)*/Y$; where $e(y)*$ is either $e(y)1^{u1}$, $e(y)2^{u1}$ or $e(y)3^{u1}$.

Construction of different combinations containing *z*, *e(y)* and *w* mutations was performed according to two schemes:

First scheme:
$F_0$ ♀ $y^2\ e(y)*/In(1)$ × ♂ $y^2\ z*\ w*\ e(y)*/Y$;
$F_1$ analysis of ♀ $y^2\ e(y)*/y^2\ z*\ w*\ e(y)*$;

where *z** is any *z* mutation, *w** is any *w* mutation, and *e(y)** is either $e(y)1^{u1}$ or $e(y)3^{u1}$.

Second scheme:
$F_0$ ♀ $y^2\ z^{v77h}w*/y^2\ z^{v77h}w*$ × ♂ $y^2\ z^{v77h}\ e(y)*/Y$;
$F_1$ analysis of phenotype of ♀ $y^2\ z^{v77h}w*/y^2\ z^{v77h}\ e(y)*$.

The combination $e(y)1^{u1}\ e(y)3^{u1}$ was obtained as follows:

$F_0$ ♀ $y^2m\ B\ e(y)3^{u1}/FM4$ × ♂ $y^2\ e(y)1^{u1}\ Bx^{eu1}/Y$;
$F_1$ ♀ $y^2m\ B\ e(y)3^{u1}/y^2\ e(y)1^{u1}\ Bx^{eu1}$ × ♂ $FM4/Y$;
$F_2$ ♀ $y^2\ e(y)1^{u1}\ e(y)3^{u1}/FM4$ × ♂ $FM4/Y$, the required strain.

Since the $e(y)1^{u1}\ e(y)3^{u1}$ combination is lethal in homozygotes (see below), this strain is permanently maintained with an *FM4* balancer.

The combination $e(y)2^{u1}\ e(y)1^{u1}$ was obtained as follows:

$F_0$ ♀ $y^2\ e(y)2^{u1}f\ Bx^{eu1}/y2\ e(y)2^{u1}f\ Bx^{eu1}$ × ♂ $y^2m\ e(y)1^{u1}/Y$;
$F_1$ ♀ $y^2\ e(y)2^{u1}f\ Bx^{eu1}/y^2m\ e(y)1^{u1}$ × ♂ $y^2m\ f/Y$;
$F_2$ ♀ $y^2\ e(y)2^{u1}\ e(y)1^{u1}/y^2m\ f$ × ♂ $FM4/Y$, the required strain.

The combination $e(y)2^{u1}\ e(y)3^{u1}$ was obtained in the same way as $e(y)1^{u1}\ e(y)2^{u1}$ combination.

**Experiments on the effect of modifiers of *zeste*:** The strains were obtained and analyzed according to the following scheme:

$F_0$ ♀ $X*/FM4$ × ♂ $S^1/Cy0,\ S^2/TM3$,

where *X** is a tested combination of mutations on the *X* chromosome; $S^1$ is either $Su(z)2^1$, $Su(z)2^4$, $Su(z)2^5$, or $Psc^1$; $S^2$ is either $Scm^{D1}$, *Su(z)302*, *Su(z)301* or $E(z)^1$.

$F_1$ analysis of phenotypic expression: ♂ $X*/Y$; $S^1/+$ or $S^2/+$.

The level of viability was calculated as the ratio of males to females in offspring from similar cross: viability = number of males with *S* mutation/number of wild-type females with *S* mutation.

TABLE 1

**Interactions between mutations in the *enhancer of yellow* loci and $z^{v77h}$ allele**

| Alleles | Eye pigmentation at 25° (18°) | | | |
|---|---|---|---|---|
| | $z^+$ | | $z^{v77h}$ | |
| | ♀ | ♂ | ♂ | ♀ |
| + | wt | wt | 10 (10) | 8 (9) |
| $e(y)1^{u1}$ | wt | wt | 5 (5) | 3 (3) |
| $e(y)2^{u1}$ | wt | wt | 8 (7) | 4 (3) |
| $e(y)3^{u1}$ | wt | wt | 4 (4) | 3 (3) |
| $e(y)4^{P1}$, $e(y)5^{P1}$, or $e(y)6^{P1}$ | wt | wt | 9 (10) | 8 (9) |

For the level of eye pigmentation (numbers in the table) here and in the following tables, see MATERIALS AND METHODS. wt, wild type.

**Estimation of eye pigmentation:** Eye color analyses were done under the dissecting microscope. Analysis of eye pigmentation was performed in 3-day-old males and females developing either at 25° or at 18° (figures in brackets): ♀ $\overline{XX}/Y$ × ♂ $X^*/Y$, analysis in males; ♀ $In(I)/X^*$ × ♂ $X^*/Y$, analysis in females; where $X^*$ is a tested combination of mutations. In each case from 50 to 100 flies were scored to determine the eye color phenotype. The following system was used for the designation of mutant *w* phenotypes: wt, wild type; 10, red eyes with brown spots; 9, brownish; 8, brown; 7, light brown; 6, dark orange; 5, orange; 4, orange yellow; 3, yellow; 2, pale yellow; 1, yellowish; 0, white eyes, or null phenotype. In particular, level 3 (yellow eyes) corresponds to that obtained in $z^1w^+/z^1w^+$ females.

Eye pigmentation was evaluated on the basis of pigmentation of the major part of its area. At the same time in many cases, some mosaicism could be observed, *i.e.*, small islands of more intensively pigmented facets, comprising about 10% of the total area. Such a moderate mosaicism is a characteristic feature of flies with the level of eye pigmentation equal to 9, 6, 5 and 4, but not of those with the level 7–8 and 3, that are more uniformly pigmented.

In a few cases, pointed out in the legends to tables, mosaicism was stronger: more intensively stained spots occupied up to 50% of all facets.

## RESULTS

**The mutations $e(y)1^{u1}$, $e(y)2^{u1}$ and $e(y)3^{u1}$ have a synergistic effect with $z^{v77h}$ on the expression of the *white* gene:** I first tested the effect of mutations in the six *e(y)* loci on the expression of *white*, in the presence of $z^{v77h}$ which results in the complete inactivation of the zeste protein (PIRROTTA *et al.* 1987).

The $z^{v77h}$ mutation itself has a very weak inhibitory action on *white* expression: the eye color is close to normal in males and brown in homozygous females (Table 1). Mutations in different *enhancer of yellow* loci alone have no effect on *white* expression. However, the combination of $z^{v77h}$ with either $e(y)1^{u1}$, $e(y)2^{u1}$ or $e(y)3^{u1}$ drastically reduces *white* gene expression, whereas its combination with mutations in the *e(y)4*, *e(y)5* or *e(y)6* loci has no effect beyond that seen with $z^{v77h}$ alone (Table 1).

The $e(y)3^{u1}$ mutation has the strongest inhibitory effect (level 4: orange yellow eyes with red spots in males) and the $e(y)2^{u1}$ mutation has the weakest effect (level 8: brown eyes in males). These effects are more prominent in females than in males. At lower temperature (18°), the inhibitory effect of some combinations of the $e(y)1^{u1}$, $e(y)2^{u1}$ or $e(y)3^{u1}$ mutations with the *z* allele is increased. The maximal inhibition reduces *white* expression to level 3 (yellow eyes, like in $z^1w^+/z^1w^+$ females) (Table 1), which is characteristic of constructions lacking the upstream enhancer controlling *white* transcription (QIAN *et al.* 1992). The residual expression after enhancer loss is known to be supported by control sequences located in the promoter region (LEVIS *et al.* 1985; PIRROTTA *et al.* 1985).

TABLE 2

**Interactions between the $e(y)1^{u1}$, $e(y)2^{u1}$ and $e(y)3^{u1}$ mutations and different *z* alleles**

| Alleles | Eye pigmentation at 25° (18°) | | | | |
|---|---|---|---|---|---|
| | $z^1$, $z^{Op6}$ or $z^{Op6R1}$ ♀ | $z^1$ ♂ | $z^{Op6}$ ♂ | $z^{Op6R1}$ ♂ | $z^{Op6R1}/z^+$ ♀ |
| + | 3 (3) | wt (wt) | 3 (5) | 6 (10) | 10 |
| $e(y)1^{u1}$ | 3 (3) | 9 (9) | 3 (3) | 4 (7) | 7 |
| $e(y)2^{u1}$ | 3 (3) | wt (wt) | 3 (3) | 7 (10) | 10 |
| $e(y)3^{u1}$ | 3 (3) | 9 (9) | 3 (3) | 3 (5) | 4 |

It should be pointed out that, in many cases, mosaicism in eye pigmentation was detected. The mosaics were estimated as darker spots on the mutant background (see MATERIALS AND METHODS). This is in general typical of several *z* mutations. Usually, mosaicism was less pronounced or even absent in eyes with pigmentation levels of 7–8 and 3, which may represent more stable levels of *white* expression. Hereafter, I will mention only cases where mosaicism was especially pronounced.

**Interaction of *enhancers of yellow* with other *zeste* alleles:** I next analyzed combinations of either $e(y)1^{u1}$, $e(y)2^{u1}$ or $e(y)3^{u1}$ with the $z^1$, $z^{Op6}$, or $z^{Op6R1}$ alleles, *i.e.*, point mutations changing the specificity of *zeste* induced interactions (Table 2). Here the effects could only be observed in males because in females, all three *z* alleles already reduce *white* expression to the lowest level (3, yellow eyes).

The $z^1$ mutation, by itself, has no effect in males (red eyes). Its combination with either $e(y)1^{u1}$ or $e(y)3^{u1}$ mildly reduces eye pigmentation (brownish eyes). In males, the partial revertant of $z^{Op6}$ (called $z^{Op6R1}$) combined with either $e(y)1^{u1}$ or $e(y)3^{u1}$ phenotypically resembles the original mutation $z^{Op6}$. Thus, $e(y)1^{u1}$ and $e(y)3^{u1}$ enhance the negative effect of *z* point mutations on *white* expression. However, the $e(y)2^{u1}$ mutation does not appear to enhance this effect.

At 25° the $z^{Op6}$ allele reduces *white* expression to level 3, even in males; the $e(y)1^{u1}$ and $e(y)3^{u1}$ mutations have no additional effect on the phenotype at this temperature. However, they do enhance the $z^{Op6}$ effect at 18° reducing pigmentation from level 5 to level 3 (yellow eyes), *i.e.*, to the level typical of an enhancerless *white*

TABLE 3

**Influence of *enhancer of yellow* mutations on the dominant effect of the $z^1$ and $z^{Op6}$ mutations**

| | Eye pigmentation at 25° (18°) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $z^1/z^+$ | | | | $z^{Op6}/z^+$ | | | |
| *w* genotype | + | $e(y)1^{u1}$ | $e(y)2^{u1}$ | $e(y)3^{u1}$ | + | $e(y)1^{u1}$ | $e(y)2^{u1}$ | $e(y)3^{u1}$ |
| $w^+/w^+$ | wt | 10 | wt | 10 | 5 (10) | 4 (8) | 7 (10) | 3 (8) |
| $w^a/w^+$ | 10 | 8 | wt | 8 | 3 (8) | 3 (7) | 4 (9) | 3 (6) |
| $w^{bf}/w^+$ | 9 | 7 | 10 | 7 | 3 (8) | 3 (6) | 3 (8) | 3 (5) |

gene. No tested combination of mutations involving the *zeste* and *e(y)1*, *e(y)2* or *e(y)3* loci can overcome this limit.

**Dominant effects of zeste in the presence of $e(y)1^{u1}$ or $e(y)3^{u1}$ mutations:** The possibility of synergistic action between heterozygous *zeste* alleles and the $e(y)1^{u1}$, $e(y)2^{u1}$ and $e(y)3^{u1}$ mutations was tested. To make the test system more sensitive, the $w^+$ allele was also used in combination with some *w* mutations ($w^a$, $w^{bf}$). The $z^1$ mutation is recessive (GANS 1953), but it has a mild dominant effect in flies with heterozygous *w* alleles. $z^{Op6}$ is a dominant allele but its effect is partially suppressed at 18° (LIFSCHYTZ and GREEN 1984). The dominant effect is almost completely suppressed in the $z^{Op6R1}$ partial revertant. Introduction of the $e(y)1^{u1}$ or $e(y)3^{u1}$ (but not the $e(y)2^{u1}$) mutations increases the dominant effect of the tested *z* alleles to some extent (Table 3). When combinate with $e(y)3^{u1}$, the $z^{Op6R1}$ mutation has a conspicuous dominant effect identical to the original allele $z^{Op6}$ (see Table 2, last column).

**Dominant effects of $e(y)3^{u1}$ on *zeste* mutations:** The mutations $e(y)1^{u1}$, $e(y)2^{u1}$ and $e(y)3^{u1}$ are recessive. However, in heterozygous condition, $e(y)3^{u1}$ significantly enhances the effect of $z^{v77h}$ on *white* expression (Table 4). Heterozygous $e(y)1^{u1}/e(y)1^+$ and $e(y)2^{u1}/e(y)2^+$ flies show no such effect. Similar results (not shown) were obtained in combinations with the $z^1$, $z^{Op6}$ and $z^{Op6R1}$ alleles. Thus, in the presence of certain *z* alleles, the $e(y)3^{u1}$ mutation becomes dominant.

**Effects of mutations in the *enhancer of yellow* loci on *white* alleles lacking zeste protein binding sites:** To investigate the nature of the regulatory elements of the *white* gene that interact with the putative e(y) proteins, I analyzed *white* alleles containing deletions or insertions in the 5′ regulatory region. The $w^{sp1}$ mutation, which results from the insertion of a roo mobile element in the *white* eye enhancer, abolishes the *zeste-white* interaction in females. The $w^{sp2}$ mutation is a partial deletion of the *white* eye enhancer, removing three of the five *zeste* binding sites (DAVISON *et al.* 1985). The $w^{sp4}$ mutation is a deletion of the complete eye enhancer plus additional 5′ sequences of the *white gene.* In $w^{sp1}$ and $w^{sp2}$, the white eye enhancer is not fully inactivated, and the level of *w* expression is still equal to levels 5 and 6, respectively (dark orange eyes, orange eyes with brown spots). A more intense eye pigmentation (level 7) in flies

TABLE 4

**Dominant effect of the $e(y)3^{u1}$ mutation**

| $z^{v77h}/z^{v77h}$ with various *w* alleles | Eye pigmentation at 25° | | |
|---|---|---|---|
| | +/+ | $e(y)1^{u1}/+$ or $e(y)2^{u1}/+$ | $e(y)3^{u1}/+$ |
| $w^+/w^+$ | 8 | 8 | 6 |
| $w^a/w^+$ | 8 | 8 | 5 |
| $w^{bf}/w^+$ | 6 | 6 | 4 |

carrying the $w^{sp4}$ allele probably may be explained by the activation of *white* gene transcription by some other more distantly located enhancer. Addition of any *z* mutation to this background does not change *white* expression further (results not shown).

The $e(y)1^{u1}$ allele has the strongest effect on these $w^{sp}$ mutations, reducing white expression to level of 2 or 3 (Table 5). The most extreme reduction occurs in the $w^{sp1}$-$e(y)1^{u1}$ combination, where the phenotype is at level 2. This reduction below level 3 (see above) may be due to additional effect of the roo element inserted in $w^{sp1}$ on *white* transcription. The $e(y)3^{u1}$ mutation also reduced pigmentation to level 3 in two of the tested cases: it has a weaker effect only in combination with the $w^{sp4}$ mutation (level 5). Thus, the effect of $e(y)3^{u1}$ is less dramatic than that of $e(y)1^{u1}$, which is opposite to the situation discussed above.

**General effects of combinations of the *z* and $e(y)1^{u1}$, $e(y)2^{u1}$ or $e(y)3^{u1}$ mutations:** The viability of *e(y)3* mutants is reduced two fold compared to $e(y)3^+$ flies. The $e(y)1^{u1}$ and $e(y)2^{u1}$ mutations do not interfere with viability. The combination of any of the *e(y)* mutants with the $z^{v77h}$ allele leads to some decrease in viability (Table 6). The $z^1$ mutation does not change the viability of the $e(y)1^{u1}$ and $e(y)3^{u1}$ mutants, whereas the $z^{Op6}$ mutation does so slightly (data not shown). In contrast, the $z^{Op6}$ and $z^1$ mutations enhance the mutant phenotype of $e(y)2^{u1}$ mutation while viability decreases. In combination with $z^{Op6}$, $e(y)2^{u1}$ gives rise to flies with spread wings, small eyes, male and female sterility and very low viability. All features of this phenotype depending on addition of $z^{Op6}$ mutation are suppressed at 18°, and the flies become identical to $e(y)2^{u1}$ single mutants.

**Effects of combinations of different *enhancer of yellow* mutations:** Combinations of mutations in two different *enhancer of yellow* loci were constructed. It was

**TABLE 5**

**Effects of the *e(y)1* and *e(y)3* mutations on the *white* alleles unmodified by *zeste* mutations**

| Alleles | Eye pigmentation at 25° in males (and females) | | |
|---|---|---|---|
| | $w^{sp1}$ | $w^{sp2}$ | $w^{sp4}$ |
| + | 5 (4) | 6 (5) | 7 (7) |
| $e(y)1^{u1}$ | 2 (2) | 3 (3) | 3 (3) |
| $e(y)2^{u1}$ | 5 (4) | 6 (5) | 7 (7) |
| $e(y)3^{u1}$ | 3 (3) | 3 (3) | 5 (5) |

**TABLE 6**

**Analysis of viability in different combinations**

| Genotype | $z^+/w^+$ | | | | $z^{v77h}w^{Bwx}$ | | | |
|---|---|---|---|---|---|---|---|---|
| | + | $e(y)$ $1^{u1}$ | $e(y)$ $2^{u1}$ | $e(y)$ $3^{u1}$ | + | $e(y)$ $1^{u1}$ | $e(y)$ $2^{u1}$ | $e(y)$ $3^{u1}$ |
| + | 1 | 1 | 1 | 0.5 | 1 | 0.5 | 0.7 | 0.3 |
| $Su(z)2^1$ | 1 | 0.4 | 0.5 | 0* | 0.7 | 0.1 | 0.2 | 0** |
| $Su(z)2^4$ | 1 | 0.7 | 0.7 | 0.2 | 1 | 0.5 | 0.5 | 0.2 |
| $Su(z)2^5$ | 1 | 0.7 | 0.7 | 0.3 | 1 | 0.5 | 0.5 | 0.3 |
| $Psc^1$ | 1 | 0.7 | 0.7 | 0.5 | 1 | 0.2 | 0.5 | 0.1 |
| $E(z)^1$ | 1 | 1 | 1 | 0* | 1 | 0.5 | 0.7 | 0** |

The figures indicate viability, *i.e.*, ratio of males to heterozygous females in corresponding combinations of mutations obtained in the crosses described in MATERIALS AND METHODS. The figures obtained were approximated to decimals. Therefore, only large differences (0.2 or more) are significant. At least, 500 females were scored if viability is higher than 0.3, at least 1000 females–if viability is lower than 0.3. In the cases indicated by asterisks, the number of scored females was 2000. * Few males (2–3) with appropriate genotype were detected; ** the males with appropriate genotype were absent.

found that two of them, $e(y)1^{u1}$ $e(y)3^{u1}$ and $e(y)2^{u1}$ $e(y)3^{u1}$, are lethal. The $e(y)1^{u1}$-$e(y)2^{u1}$ males have very low viability and are completely sterile. The $e(y)1^{u1}$ mutation also enhances all phenotypic effects induced by the $e(y)2^{u1}$ allele.

**Analysis of combinations of $e(y)1^{u1}$, $e(y)2^{u1}$ or $e(y)3^{u1}$ mutations with modifiers of *zeste*:** Mutations in several genes are known either to enhance or suppress the *zeste-white* interaction. We have combined these mutations with mutations in the *e(y)* loci. The $z^1w^{zm}$ strain was used as a test system, as the action of $z^1$ allele in the presence of $w^{zm}$ is realized even in males (PETERSON *et al.* 1994).

Mutations in the *e(y)1* and *e(y)3* genes reduce eye pigmentation in this strain down to levels 5 and 4, respectively. The characteristic feature is a strong mosaicism, with large red and brown spots present in the eyes. $E(z)^1$ has a similar effect: reduction of pigmentation in the $z^1w^{zm}$ strain down to level 3, accompanied by strong mosaicism. Combinations of either the $e(y)1^{u1}$ or $e(y)3^{u1}$ mutations with $E(z)^1$ do not change the phenotype obtained in the presence of $E(z)^1$ alone (Table 7). Interestingly, the combination of mutation $e(y)3^{u1}$ and $E(z)^1$ is practically lethal (Table 6). The $e(y)2^{u1}$ mutation slightly suppresses the $z^1w^{zm}$ phenotype and does not change the effect of $E(z)^1$ (Table 7).

**TABLE 7**

**Eye pigmentation in combinations of mutations in the *e(y)* loci and mutations modifying the *zeste-white* interaction**

| Genotypes | $z^{v77h}w^{Bwx}$ | | | | $z^1w^{zm}$ | | | |
|---|---|---|---|---|---|---|---|---|
| | + | $e(y)$ $1^{u1}$ | $e(y)$ $2^{u1}$ | $e(y)$ $3^{u1}$ | + | $e(y)$ $1^{u1}$ | $e(y)$ $2^{u1}$ | $e(y)$ $3^{u1}$ |
| + | 7 | 5 | 6 | 4 | 7 | 5[a] | 9 | 4[a] |
| $E(z)^1$ | 7 | 5 | 6 | | 3 | 3 | 3[a] | 3 |
| $Su(z)2^1$ | 7 | 5–6 | 6–7 | | wt | wt | wt | wt |
| $Su(z)2^4$ | 7 | 5 | 6 | 4 | wt | 10 | wt | 10 |
| $Su(z)2^5$ | 7 | 5–6 | 6 | 4 | wt | 10 | wt | 10 |
| $Psc^1$ | 7 | 5–6 | 6 | 5 | wt | 10 | wt | 10 |
| $Su(z)301$ | 7 | 5 | 6 | 4 | wt | 9 | wt | 8 |
| $Su(z)302$ | 7 | 5 | 6 | 4 | wt | 9 | 10 | 7 |
| $Scm^{D1}$ | 7 | 5 | 6 | 4 | wt | 9 | 10 | 7 |

[a] Strong mosaicism.

Other mutations analyzed, $Su(z)2^1$, $Su(z)2^4$, $Su(z)2^5$, $Psc^1$, $Su(z)301$, $Su(z)302$ and $Scm^{D1}$, suppress the mutant *w* phenotype. They either partially or completely suppress the *w* phenotype in combination with $z^1w^{zm}$ and the *e(y)* alleles. However, these modifiers of *zeste* do not interfere with $z^{v77h}$. They do not change the phenotype of $z^{v77h}w^+$ and $z^{v77h}w^{Bwx}$ combinations. At the same time, they do not change the effect of mutations in the *e(y)* genes on these combinations. Only strong suppressors ($Su(z)2^1$, $Su(z)2^5$ and $Psc^1$) slightly suppress the *w* phenotype in *e(y)* backgrounds (Table 7).

$Scm^{D1}$, $Su(z)302$ and $Su(z)301$ mutations do not influence the viability of the $e(y)1^{u1}$, $e(y)2^{u1}$ or $e(y)3^{u1}$ alleles, or their combinations with $z^{v77h}$, $z^1$ or $z^{Op6}$ mutations (not shown). The $Su(z)2^1$ mutation has the strongest effect on viability of above mentioned alleles. Other *Su(z)2* alleles and the $Psc^1$ mutation have a weaker effect. Combinations of $e(y)3^{u1}$ with $Su(z)2^1$ or $E(z)^1$ alleles give rise to high levels of lethality (Table 6).

## DISCUSSION

**Relationship between enhancement of the *zeste-white* interactions and other effects of the *e(y)1*, *e(y)2* and *e(y)3* mutations:** The *e(y)1*, *e(y)2* and *e(y)3* genes belong to a family of regulatory genes that have similar and overlapping functions in development. Mutations in these genes have pleiotropic effects. In particular, in combination with some *y* alleles, they influence *yellow* gene expression in bristles and some other cuticle derivatives (GEORGIEV and GERASIMOVA 1989) and sometimes in all areas of the cuticle (P. G. GEORGIEV, unpublished). $e(y)1^{u1}$ females are completely sterile, whereas $e(y)3^{u1}$ causes only reduced fertility and viability. The $e(y)2^{u1}$ mutation partially suppresses the $f^1$ allele induced by a *gypsy* insertion within an intron of the *forked* gene (HOOVER *et al.* 1993), and mutations in the *e(y)1* and *e(y)3* loci (but not in *e(y)2*) partially suppress the $sc^{D1}$ mutation (P. G. GEORGIEV, unpublished). The $e(y)2^{u1}$ mutation also strongly changes the general

morphology of the fly: the body is shortened, the wings are spread, and the eyes are small (GEORGIEV and GERASIMOVA 1989). These results, taken together, suggest that the *e(y)1*, *e(y)2* and *e(y)3* genes may play an important role in the control of the expression of several genes.

The results presented here demonstrate that this group of genes (*enhancer of yellow 1*, *2* and *3*) also interacts with *zeste*, and that their putative protein products are able to compensate for the absence of zeste protein in the activation of *white* transcription. In contrast to all other modifiers of *zeste*, mutations in the *e(y)1*, *e(y)2* and *e(y)3* genes interact with the $z^{v77h}$ mutation, a null *zeste* allele. Thus, the protein products of these three genes may have some *zeste*-like functions and compensate for the absence of zeste protein.

What is the relationship between the observed phenomena and earlier described effects of mutations in the *e(y)1*, *e(y)2* and *e(y)3* loci? We have recently found that the enhancing effects of $e(y)1^{u1}$, $e(y)2^{u1}$ and $e(y)3^{u1}$ mutations on mutant *yellow* phenotypes depend on the presence of foreign sequences within the *yellow* locus, even if these sequences do not change *yellow* expression by themselves. Moreover, the $z^{v77h}$ allele has been found to enhance the effects of *enhancer of yellow* mutations, especially of $e(y)1^{u1}$ and $e(y)3^{u1}$, on *yellow* expression (P. G. GEORGIEV and A. KULIKOV, in preparation). The latter observation is reminiscent of the results obtained with the *white* locus.

**Possible role of e(y)1, e(y)2 and e(y)3 proteins:** Zeste is a DNA-binding protein that might play a role in enhancer-promoter interactions (see *Introduction*). Similar functions are suggested for the putative e(y)1, e(y)2 and e(y)3 proteins as they seem to compensate for the absence of zeste protein and, when mutant, *white* expression is reduced to a level characteristic of an enhancerless *white* gene. Recently, a special anchor site has been identified that lies immediately upstream of the *white* gene in the promoter area. This site plays a role in mediating interactions between enhancers and the promoter (QIAN *et al.* 1992). We hypothesize that the e(y) proteins might bind to this anchor site. When zeste protein binding sites in the enhancer are destroyed or deleted ($w^{sp}$ mutations), e(y) proteins begin to play a dominating role in contacts between enhancer and promoter.

The *e(y)3* gene may play a key role, since mutations in this gene significantly enhance the effect of $z^{v77h}$ even in heterozygotes, although qualitatively the effects of the $e(y)3^{u1}$ and $e(y)1^{u1}$ mutations are similar. In contrast to $e(y)1^{u1}$ and $e(y)3^{u1}$, the $e(y)2^{u1}$ mutation does not influence the phenotype of $w^{sp}$ mutations. An enhancement of all features of the $e(y)2^{u1}$ phenotype, including decreased female fertility, takes place in combinations with $z^{Op6}$ or $z^{1}$. These features disappear at 18°. It is known that the $z^{Op6}$-*white* interaction is partially suppressed at 18°, possibly as a result of conformational changes in the mutant zeste protein. These data suggest a closer relationship between e(y)2 and zeste proteins: either their direct interaction in the formation of enhancer-promoter contacts or independent binding to similar or closely located sites.

As has been mentioned above, *zeste* point mutations that change the specificity of zeste protein interactions inhibit *white* gene expression to a larger extent than *zeste* null alleles. The presence of mutant zeste protein inhibits the interaction of the eye enhancer with the promoter. Instead, the enhancer contacts other sequences located in the homologous or even in the same chromosome (in the case of strong *z* alleles). Thus, the enhancer becomes isolated from the promoter (WU *et al.* 1989). The $z^{Op6}$ and $z^{1}$ mutations are active only in the presence of *zeste* protein binding sites in the eye enhancer (QIAN *et al.* 1992). In combinations with these alleles, mutations in the *e(y)1* and *e(y)3* loci have proportionally weaker effects, as the level 3 of *white* expression is reached even in some *z* mutants. Nevertheless, sometimes a significant further reduction of *white* gene expression takes place. There may be a competition between e(y)1 and e(y)3 proteins and mutant zeste protein for the formation of correct or alternative contacts which depends on the strength of the *z* mutation. In contrast, the $e(y)2^{u1}$ mutation usually does not interfere with *z* point mutations or even slightly suppresses them. This may be explained by the interaction of their protein products.

**Differences between the *e(y)1*, *e(y)2* and *e(y)3* genes and known modifiers of *zeste*:** Mutations in five other genes have been found to modify the effects of the $z^{1}$ mutation: *Su(z)2*, *Su(z)3*, *Psc*, *Su(z)301/E(z)1/pco*, and *Su(z)302/Scm* (WU *et al.* 1989; JONES and GELBART 1990; PHILLIPS and SHEARN 1990). Different alleles of the same locus can either enhance or suppress *zeste*-dependent transvection effects. The *Suppressor* and *Enhancer of zeste* mutations are dominant modifiers of transvection phenomena and lethal as homozygotes. At least three of the loci are members of the *Polycomb* group, which regulates the expression of the bithorax complex and antennapedia complex (JURGENS 1985). A possible function for *Pc* gene products might be to package chromatin into higher order structures (PIRROTTA 1990; PARO and HOGNESS 1991).

Mutations in these genes influence only *zeste*-dependent transvection, but not direct enhancer-promoter contacts in the *white* locus, *i.e.* they do not interfere with the $z^{v77h}$ null allele. Thus, the protein products of these genes seem to modify the interaction of the mutant zeste protein, rather than to compensate *zeste* function in *zeste* null alleles, as e(y)1, e(y)2 and e(y)3 proteins do. Their products are therefore good candidates for proteins that directly interact with the zeste protein (PIRROTTA 1990). Another possibility is that modifiers of the *zeste-white* interaction change the chromatin conformation, and in this way, modify the efficiency of zeste protein binding to DNA.

Modifiers of the $z^1$ mutation do not influence the cooperative effect of mutations in the *e(y)* loci with *zeste* null alleles and mutations in the *e(y)* loci have very low if any effect on the interaction between modifiers and the $z^1$ allele. The easiest explanation is that the effect of modifiers is realized indirectly through changes in the level of chromatin compaction, possibly in certain areas of the genome. It is more difficult to explain the synthetic lethality produced by the combinations $E(z)^1;e(y)3^{u1}$ and $Su(z)2^1;e(y)3^{u1}$. This suggests a direct interaction between the two genes.

The genetic experiments presented in this study suggest an important role for the *e(y)1*, *e(y)2* and *e(y)3* genes in the organization of long distance interactions in chromosomes.

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