Proc. Natl. Acad. Sci. USA
Vol. 76, No. 3, pp. 1368–1372, March 1979
Genetics

Allelic pairing and gene regulation: A model for the zeste–white interaction in *Drosophila melanogaster*

(repressor/nuclear architecture/transvection)

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Communicated by Dan L. Lindsley, December 11, 1978

ABSTRACT **Data are presented that indicate that the *z* locus of *Drosophila melanogaster* represses *w* locus activity, but the repression is effective only on paired or physically adjacent *w* loci. Various mutant alleles of *z* and *w* were combined in a series of different doses to determine the effect of dosage and physical position in the nucleus on gene expression. In *z*/*z* individuals, paired white alleles fail to be expressed, while unpaired alleles are expressed normally. The results are discussed in terms of a model postulating that the *z* gene product represses the transcription of w^+ by complexing with an RNA produced by part of the white locus itself. In order to be effective in repression, there must be two w^+ genes producing the RNA in a limited volume in the nucleus. Such a model necessarily imposes a specific architecture on the chromatin of interphase nuclei.**

Examples of regulatory interactions between loci in eukaryotes are rare; communication between two alleles at a single locus is even less well documented. We present evidence that a particular locus of *Drosophila melanogaster* functions as a repressor of another locus. Repression is generally apparent, however, only if two alleles of the repressed locus are physically adjacent or paired.

Gans (1) discovered the mutation zeste (*z*; 1–1.0) that produces yellow eye color in females but is wild type in males. She showed that the expression of the zeste phenotype in the females is due to the presence of two wild-type alleles of the white locus (*w*; 1–1.5). Certain duplications of w^+ allow zeste phenotype expression in males. On the other hand, females heterozygous for a white locus deletion ($z\ w^+/z\ w^-$) are wild type in eye color. Some mutant alleles of *w* act in the same fashion as a deletion of the locus (1). Such white locus alleles were determined by Green (2) to map in the proximal part of the white locus (here designated as w^{prx}). Those alleles of white located in the distal portion of the locus (w^{dst}) allow expression of zeste just as w^+ does. In fact, all the mutations that appear to upset regulatory function of the white locus map in w^{prx}. Pattern mutants that produce mosaics of pigmented and nonpigmented facets are located in w^{prx}, and all mutants in the region fail to show dosage compensation, which is characteristic of w^+. A tandem duplication of only the w^{prx} portion of the locus behaves with respect to *z* as two doses of w^+ (3). On the basis of these and some other distinguishing properties, Judd (4) has suggested that w^{prx} is involved in the regulation of the white locus while w^{dst} contains the structural sequence.

Historically the zeste–white interaction has been stated in terms of white locus deletions and mutants in w^{prx} acting as dominant suppressors of zeste. We believe this is not an accurate description of the system, and we will here describe the interaction from the point of view of repression of w^+ by *z*. The reduction in pigmentation in $z\ w^+/z\ w^+$ females results from the continued repression of w^+ even when the gene should be active. The reason the $z\ w^+/Y$ male is wild type is that two doses of w^+ are necessary before repression by the mutant *z* can be observed. Indeed, certain duplications of w^+ in *z* males will be repressed and a zeste phenotype will be produced. This is not true for all duplications of w^+, however. Gans noted that translocations of w^+ to new positions in the genome, when used as duplications, allow w^+ to be expressed. Gelbart (5) confirmed that some w^+ translocations produce wild-type eye pigmentation in homozygous *z* flies, a phenomenon he termed "ultrasuppression." It became clear to us that the zeste–white interaction depends not only on the dosage relationships of the two loci but also on some aspect of the position of the w^+ alleles within the two sets of chromosomes of the diploid nucleus.

We have used insertional translocations and various alleles of *z* and *w* to clarify the nature of positional and dosage relationships. From the information provided by these experiments we have constructed a model that accounts for the zeste and white loci interactions and that may have more general application in understanding chromosome organization and gene regulation in eukaryotes.

MATERIALS AND METHODS

The approach to determining the mechanism of interaction between the zeste and white loci was to construct individuals with genotypes that differed in the dosage of the *z* and *w* loci, with the positions of these different doses, particularly for *w*, differing as well. This allowed the determination of which of the *w* loci were being repressed and what the relationship between loci and between alleles must be for normal function of these genes. We combined various white alleles in the X chromosomes with insertional translocations of the white locus. Both one and two doses of the white locus were used at both the translocated and nontranslocated positions to find out which alleles were active with various arrangements of the genes. Alleles were chosen so that we could distinguish between the activity of the two sets located in different parts of the genome. Thus, we were able to ascertain the effect of gene dose and position on the zeste–white interaction.

The white alleles used were w^+, w^{Bwx}, w^{col}, and w^{11E4}. w^{Bwx}/w^{Bwx} females and w^{Bwx} males have brown eye color, and w^{col} eye color is a dark brownish-red in both sexes. Both w^{Bwx} and w^{col} are similar to w^+ in that the $z\ w^{Bwx}$ and $z\ w^{col}$ homozygous females are zeste, while hemizygous males show no zeste effect. w^{Bwx} has an additional advantage of being mildly dominant, with w^{Bwx}/w^+ flies having slightly darker eyes than wild type. w^{11E4} gives pure white eyes and is the equivalent of a white deletion.

The duplications used were $Dp(1;2)w^{+51b7}$, $Dp(1;3)$-$N^{264-58a}$, $Dp(1;3)w^{49a7}$, and $Dp(1;3)w^{zh}$. $Dp(1;2)w^{+51b7}$ is a nonvariegating, wild-type insertional translocation of the white locus and some adjacent genes into chromosome 2. The

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Table 1. Phenotypes of flies with autosomal white locus duplications and without a functional white gene at 3C

Genotype	Phenotype
$z\ DF(1)w^{zh}/z\ Df(1)w^{zh};\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Zeste with halo
$z\ Df(1)w^{zh}/Y;\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Zeste with halo
$z\ w^{11E4}/z\ w^{11E4};\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Zeste with halo
$z\ w^{11E4}/Y;\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Zeste with halo
$z\ Df(1)w^{zh}/z\ Df(1)w^{zh};\ Dp(1;3)w^{zh}$	Wild type
$z\ w^{11E4}/z\ w^{11E4};\ Dp(1;2)w^{+51b7}/Dp(1;2)w^{+51b7}$	Zeste
$z\ w^{11E4}/Y;\ Dp(1;2)w^{+51b7}/Dp(1;2)w^{+51b7}$	Zeste
$z\ w^{11E4}/z\ w^{11E4};\ Dp(1;2)w^{+51b7}$	Wild type
$z\ w^{11E4}/Y;\ Dp(1;2)w^{+51b7}$	Wild type
$z\ w^{11E4}/z\ w^{11E4};\ Dp(1;3)w^{49a7}/Dp(1;3)w^{49a7}$	Zeste with red spots
$z\ w^{11E4}/Y;\ Dp(1;3)w^{49a7}/Dp(1;3)w^{49a7}$	Zeste with red spots
$z\ w^{11E4}/z\ w^{11E4};\ Dp(1;3)w^{49a7}$	Red and white variegated
$z\ w^{11E4}/Y;\ Dp(1;3)w^{49a7}$	Red and white variegated
$z\ w^{11E4}/z\ w^{11E4};\ Dp(1;3)N^{264-58a}/Dp(1;3)N^{264-58a}$	Zeste with red spots
$z\ w^{11E4}/Y;\ Dp(1;3)N^{264-58a}/Dp(1;3)N^{264-58a}$	Zeste with red spots
$z\ w^{11E4}/z\ w^{11E4};\ Dp(1;3)N^{264-58a}$	Red and white variegated
$z\ w^{11E4}/Y;\ Dp(1;3)N^{264-58a}$	Red and white variegated
$z^{+}\ w^{11E4}/z^{+}\ w^{11E4};\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Wild type
$z^{+}\ w^{11E4}/z^{+}\ w^{11E4};\ Dp(1;2)w^{+51b7}/Dp(1;2)w^{+51b7}$	Wild type
$z^{+}\ w^{11E4}/z^{+}\ w^{11E4};\ Dp(1;3)w^{49a7}/Dp(1;3)w^{49a7}$	Red and white variegated
$z^{+}\ w^{11E4}/z^{+}\ w^{11E4};\ Dp(1;3)N^{264-58a}/Dp(1;3)N^{264-58a}$	Red and white variegated

breakpoints are 3C1-2, 3D6, and 52F on the polytene chromosome map of Bridges (6). $Dp(1;3)N^{264-58a}$ is an insertion of 3B2-4 to 3D5-6 into 80D-F. $Dp(1;3)w^{49a7}$ inserts 3A9-B2; 3E2-3 into 81. Both $N^{264-58a}$ and w^{49a7} variegate for w^{+}. $Dp(1;3)w^{zh}$ is an insertion of 3C2-3C6 into 61D. The duplication carries wild-type alleles of white, roughest, and verticals. It will be designated as $Dp\ w^{zh}$. The X chromosome from which this segment was removed is designated as $Df(1)w^{zh}$. Females of genotype $z\ Df(1)w^{zh}/z\ Df(1)w^{zh};\ Dp\ w^{zh}$ are wild type, as are $z\ Df(1)w^{zh}/Y;\ Dp\ w^{zh}$ males. On the other hand, $z\ Df(1)w^{zh}/z\ Df(1)w^{zh};\ Dp\ w^{zh}/Dp\ w^{zh}$ females and $z\ Df(1)w^{zh}/Y;\ Dp\ w^{zh}/Dp\ w^{zh}$ males have halo eyes, zeste in the center with red pigment on the periphery. This unusual phenotype is equivalent in the z and w interaction to a zeste phenotype. For origin of $Dp(1;3)w^{zh}$ and its expressions and behavior as a transposable element, consult Judd (4). $Dp(1;1)w^{rdp}$ is a tandem duplication of w^{prx} containing the mutation w^{bf} in w^{dst}. $Dp(1;1)w^{rdp+}$ is the same but without the w^{bf} mutation. A tandem duplication of z, $Dp(1;1)z^{59d15}$ was also used. The duplication includes 2F5-3A1 to 2A4-5 and contains the z allele but does not contain the white gene. The translocations and white alleles are described in more detail by Lindsley and Grell (7).

RESULTS

The first clue to the basis of the zeste and white loci interaction was the observation that although wild-type pigmentation is produced in a homozygous z fly carrying an insertional translocation of w^{+}, zeste pigmentation results when the translocation is made homozygous. The expression of zeste apparently is dependent not on the dosage of w^{+} alone but on the positions of the w^{+} genes relative to each other in the nucleus. Tables 1 and 2 give the phenotypes of a series of genotypes involving different doses of z and w^{+} with the positions of w^{+} varying relative to each other. The zeste phenotype is, of course, expressed in $z\ w^{+}/z\ w^{+}$ females in which w^{+} occupies its normal X chromosome position at 3C2. Also, in $z\ Df(1)w^{zh}/z\ Df(1)w^{zh};\ Dp\ w^{zh}/Dp\ w^{zh}$, in which w^{+} has been removed from the X and inserted into the left arm of chromosome 3, zeste is expressed. If, however, one of the w^{+} genes is located in X and the other in 3L ($z\ w^{+}/z\ Df(1)w^{zh};\ Dp\ w^{zh}$), the eye color is wild type. When we put w^{+} genes at 3C and another location, a homozygous z fly showed the zeste phenotype only when two doses of w^{+} were present at both the 3C and translocated positions. For example, $z\ w^{+}/z\ w^{+};\ Dp\ w^{zh}/Dp\ w^{zh}$ individuals are zeste, while $z\ w^{+}/Y;\ Dp\ w^{zh}/Dp\ w^{zh}$ and $z\ w^{+}/z\ w^{+};\ Dp\ w^{zh}$ individuals are wild type. As shown in Table 1, the other duplications behave the same way, but with minor variations due to variegation of $N^{264-58a}$ and w^{49a7}.

Table 2. Phenotypes of z/z or z/Y flies with functional white genes on both the X and third chromosomes

Genotype	Phenotype
$z\ w^{+}/z\ w^{+};\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Zeste with halo
$z\ w^{+}/Y;\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Wild type
$z\ w^{+}/Y;\ Dp(1;3)w^{zh}$	Wild type
$z\ w^{+}/z\ w^{+};\ Dp(1;3)w^{zh}$	Wild type

Apparently, the zeste phenotype can be expressed whenever a w^{+} allele is capable of pairing or is closely juxtaposed with another w^{+}. The proximity of w^{+} to z is not critical. We hypothesize that the zeste phenotype of z/z or z/Y individuals is due to the repression of paired white loci but that any unpaired white locus will be expressed in a homozygous z fly. To test the hypothesis, we constructed genotypes that would distinguish the expression of white alleles at 3C from white alleles translocated to other positions in the genome. For instance, w^{Bwx} expression gives brown eye color, easily distinguishable from the wild-type expression of w^{+51b7}. In Table 3, we see that $z\ w^{Bwx}/Y;\ w^{+51b7}/w^{+51b7}$ males are light brown (brownex + zeste), while $z\ w^{Bwx}/z\ w^{Bwx};\ w^{+51b7}$ females are wild type. $z\ w^{Bwx}/Y;\ w^{+51b7}$ males have dark brown eyes characteristic of the w^{Bwx}/w^{+} heterozygote, showing that both of the unpaired alleles are expressed. The w^{col} allele and $Dp\ w^{zh}$ were used in similar experiments to demonstrate that the phenomenon is not allele specific. Those results are also given in Table 3.

W. M. Gelbart (unpublished results) has tested various duplications in homozygous z flies. He used $Dp(1;2)w^{+70h}$ (3A7-8;3C2-3;31A3) and $Dp(1;3)w^{+67K27}$ (3A4-5;3E8-F1;

Table 3. Phenotypes of flies with white alleles positioned at 3C that are distinguishable from the white alleles translocated to autosomal positions

Genotype	Phenotype
$z\ w^{Bwx}/z\ w^{Bwx}$	Zeste
$z\ w^{Bwx}/Y$	Brown
$z\ w^{Bwx}/z\ w^{Bwx};\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Zeste with halo
$z\ w^{Bwx}/Y;\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Brown with wild-type halo
$z\ w^{Bwx}/z\ Df(1)w^{zh};\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Brown with wild-type halo
$z\ w^{Bwx}/z\ w^{Bwx};\ Dp(1;3)w^{zh}$	Wild type
$z\ w^{Bwx};Y;\ Dp(1;3)w^{zh}$	Darker wild type
$z\ w^{Bwx}/z\ w^{Bwx};\ Dp(1;2)w^{+51b7}/Dp(1;2)w^{+51b7}$	Zeste
$z\ w^{Bwx}/Y;\ Dp(1;2)w^{+51b7}/Dp(1;2)w^{+51b7}$	Light brown (brownex + zeste)
$z\ w^{Bwx}/Y;\ Dp(1;2)w^{+51b7}$	Darker wild type
$z\ w^{col}/z\ w^{col}$	Light zeste
$z\ w^{col}/Y$	Red
$z\ w^{col}/z\ w^{col};\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Zeste with halo
$z\ w^{col}/Y;\ Dp(1;3)w^{zh}/Dp(1;3)w^{zh}$	Red with wild-type halo
$z\ w^{col}/z\ w^{col};\ Dp(1;3)w^{zh}$	Wild type
$z\ w^{col}/Y;\ Dp(1;3)w^{zh}$	Wild type

86E17) in addition to the duplications we tested. His results confirm ours and show also that any two duplications heterozygous in z/z flies give wild-type pigmentation. The only exception is that $z\ w^{11E4}/z\ w^{11E4};\ Dp^{N264\text{-}58a};\ Dp\ w^{m49a7}$ females are identical to duplication homozygotes. Because both of these duplications are located in third chromosome heterochromatin, they are apparently brought close enough together in the heterozygote to be repressed by z. We conclude that paired or juxtaposed white loci are repressed by the z locus action, but any unpaired w^+ allele is active independently of paired alleles.

Knowing that increasing the dosage of w^{prx} would increase the repressor activity of z in z^+/z heterozygotes, we wanted to test the effect of increasing z dosage while holding w^+ dose constant. We compared z^+/z heterozygotes to $z^+/Dp(1;1)z^{59d15}$ heterozygotes. Table 4 shows the results. The $z^+/Dp\ z$ flies have less pigment than z^+/z when two doses of w^+ are present. Also, in flies with three doses of w^+, $z^+/Dp\ z$ gives lighter eye color than z^+/z.

DISCUSSION

In light of these results, we propose that z inhibits the transcriptional activity of the white gene. In support of this claim, consider the nature of the zeste gene itself. The alleles of zeste can be divided into three classes: normal function (z^+), modified function (e.g., z), and null (e.g., z^a). The z^a alleles must be null or at least hypomorphic. Like a z deficiency, the z^a alleles give the phenotype of whatever allele they are heterozygous with (Table 5) (8). Only the homozygous zeste-deficient condition has not been tested because the available deficiencies uncover lethal genes in the zeste region. z cannot be null because it behaves unlike z^- (8), and by the same argument z^+ cannot be null. Thus, z^a is the only known type of zeste allele that can possibly be null. Moreover, a null allele should be more easily induced than an allele with altered function, and z^a alleles are, in fact, more easily induced than z alleles. Of six nonrearranged zeste mutants that Gelbart (5) induced and tested, five were z^a types and one behaved as a weak z mutant.

Table 4. Phenotypes of individuals with different doses of z, z^+, and w^+

Genotype	Phenotype
$z^+\ w^+/z\ w^+$	Wild type
$z^+\ w^+/Dp(1;1)z^{59d15}\ w^+$	Orange mottled
$z\ w^+/z^+\ Dp(1;1)w^{rdp+}$	Light orange mottled
$z^+\ Dp(1;1)w^{rdp+}/Dp(1;1)z^{59d15}\ w^+$	Zeste

Table 5. Combinations of zeste alleles

Genotype	Phenotype
z/z	Zeste
z/z^a	Zeste
z/z^+	Wild type
z^a/z^a	Wild type
z/z^- *	Zeste
z^+/z^- *	Wild type
z^+/z^a	Wild type
z^a/z^- *	Wild type

* z^- symbolizes a deficiency for the z locus. These data are compiled from Gans (1) and results have been confirmed by our own experiments.

If we are correct in assuming that z^a is null, the z^+ function is not necessary for wild-type pigmentation because z^a homozygotes and hemizygotes are wild type. Thus, z does not reduce pigmentation simply by failure to produce z^+ product, but rather the z allele must actively interfere with some step in the pigmentation process. What step? We have shown that with z homozygous and with w^+ genes located at both the 3C and a translocated position in the same genome, only unpaired white alleles are fully expressed, while paired alleles are not. In all likelihood, zeste does not block white locus expression post-transcriptionally, because then the interfering agent would have to distinguish the product of paired from that of unpaired alleles. That would imply that both paired and unpaired alleles are active, but that the products are different. The most likely explanation, therefore, is that z causes repression of the transcription of paired w^+ alleles, although the actual repressor need not necessarily be the direct product of zeste.

Interestingly, homozygous z^a also enhances the mutant phenotype of some bithorax alleles (8). However, z does not enhance bithorax, and z^+/z^a and z/z^a heterozygotes also fail to enhance. Varying the white alleles has no effect on the ability of z^a to enhance. Kaufman *et al.* (8) conclude that the enhancement of the bithorax phenotype by z^a is probably caused by a difference in the level of some metabolite in the z^a flies. This explanation is reasonable, but the data do not rule out other kinds of effects.

The interaction of the white alleles in being repressed appears to be a form of the transvection phenomenon noted by Lewis (9). He suggested that pairing of bithorax alleles must be important to that gene's function, because heterozygous rearrangements with breakpoints near the bithorax locus enhance the mutant phenotype of some mutant combinations in the *trans* configuration. Examples of asynapsis affecting a gene's function have been described by Ashburner (10, 11) and Korge (12). In these latter cases, one strain of flies with a particular gene active, as evidenced by a puff at a locus in the polytene chromosomes of the salivary glands, was crossed to another strain with the same gene inactive. In the hybrids both alleles are normally active. However, in studying the 64C puff gene, Ashburner (10) found that when the chromosomes were unpaired in the 64C region in a particular hybrid nucleus, one chromosome puffed in the 64C region while the other remained

condensed. On the other hand, Korge (12) found that at 3C11, puffs formed on both chromosomes of hybrids except when the chromosome with the inactive 3C11 puff was heterozygous with the multiply inverted chromosome FM6. Then the FM6 chromosome puffed, but the other did not. Korge's work is particularly instructive because when a puff is induced at 3C11 in a strain that does not normally puff there, the gene activity can be correlated with the presence of a distinctive glue protein.

It appears that if a duplicated w^+ can pair with the normally positioned w^+ of a z male, then both w^+ genes will be repressed. Gans (1) found that in $z\ w^+$ males the $z\ w^+$ duplications $(Dp(1;3)z^7$ (3D3-4; 100D1-2) and $Dp(1;f)z^9$ (3E7-F1; 19-20) give zeste eye color. Both of these duplications are observed to pair with the X chromosome in polytene chromosome preparations. As we have already mentioned, if the additional w^+ gene is carried in an insertional duplication in which pairing is not possible, the males will have wild-type eyes. This supports the notion that the w^+ alleles must be paired in order to be repressed by z.

Duplications that show a variegated position effect on the white locus produce an unexpected phenotype when homozygous in z/z flies. Genotypes $z\ w^{11E4}/z\ w^{11E4}$ with either $Dp(1;3)w^{49a7}$ or $Dp(1;3)N^{264\text{-}58a}$ homozygous produce an eye color that is zeste with small spots of wild type (Table 1). This is unexpected because those cells in which the white locus is not active in either copy of the duplication should be white. Those cells with one white locus active should be wild type, and those cells in which both copies are functional should have repressed w^+ genes, producing zeste eye color. The lack of white sections in the eyes of these genotypes could be explained if the production of zeste pigment were nonautonomous. However, because white locus activity is autonomous and because Becker (13) has induced mosaic zeste and white spots by somatic exchange, we assume that the zeste coloration is also autonomous.

Because the activity of z is so specific as to block transcription of a particular gene, it seems unlikely that a single mutation of z^+ could have produced a neomorphic activity such as this *de novo*. Rather, the repression of w^+ by z is probably a variant of z^+ activity. The z and z^+ alleles appear to be antagonistic toward each other; therefore, z is most probably a modified form of z^+. In a z/z^+ heterozygote, w^+ alleles are partially repressed if three doses of w^{prx} are present in close proximity to one another, but no repression occurs with only two doses of w^{prx}. The two alleles z and z^+ seem to compete with one another. The key to the nature of this competition is provided by the observation that added doses of w^{prx} in z/z^+ heterozygotes actually reduce the amount of eye pigmentation. If the z^+ and z products were competing for a repressor site in w^{prx}, an increased number of sites should produce a more normal phenotype. That z is dominant to z^+ when three or more doses of w^{prx} are present leads us to believe that some product of w^{prx} is required by the products of z and z^+ for their activation. We postulate that the competition between z and z^+ is for an activator that is generated by the white locus itself. Higher dosages of w^{prx} provide higher levels of activator, leading to greater repression of w^+ activity.

In $z\ w^+/Y$ males the amount of activated z product is so low that no reduction of w^+ activity is apparent. However, the fact that $z\ w^a/Y$ males are reduced to an ivory eye color from the orange-pink of $z^+\ w^a/Y$ males is evidence that z has repressor activity in males but at such a low level as to go unnoticed with w^+ or the dark colored alleles. On the other hand, two doses of w^{prx} in close proximity to one another are sufficient to produce a high enough concentration of activator for z to fully repress w^+.

Table 6. Calculation of the amount of activator available to z product, based on a model assuming that products of z and z^+ compete equally well for an activator produced by w^+

Genotype	Fraction of zeste loci occupied by z	Number of w^+ loci	w^+ product available to z product	Phenotype
$z\ w^+/Y$	1	1.0	1.0	Wild type
$z\ w^+/z\ w^+$	1	2.0	2.0	Zeste
$z\ Dp\ w^+/Y$	1	2.0	2.0	Zeste
$Dp\ z\ w^+/z^+\ w^+$	2/3	2.0	1.33	Orange mottled
$z\ Dp\ w^+/z^+\ w^+$	1/2	3.0	1.5	Light orange mottled
$Dp\ z\ w^+/z^+\ Dp\ w^+$	2/3	3.0	2.0	Zeste
$z\ Dp\ w^+/z^+\ Dp\ w^+$	1/2	4.0	2.0	Zeste

In this table $Dp\ w^+$ refers to $Dp(1;1)w^{rdp+}$, and $Dp\ z$ refers to $Dp(1;1)z^{59d15}$.

In z/z^+ heterozygotes, the amount of activator available to z product molecules is proportional to the ratio of z genes to z^+ in the genome. If, for example, we suppose that z and z^+ products compete equally, we can calculate the amount of activator available to the z product when various doses of w^{prx} are present. Such a calculation is given in Table 6. If the amount of w^+ product available to z is one dose or less, little or no repression occurs and the phenotype is wild-type eye color. A dose level of two w^+ produces a zeste phenotype, and levels between one and two give eye colors intermediate between zeste and wild type.

We do not know what the postulated activator is, but we do know that the deletion of w^{prx} or mutations in w^{prx} remove the z activator function. We also know that close proximity of w^+ alleles is necessary for the w^{prx} dosage to be effective. On this basis we hypothesize that the activator of the zeste locus product is an RNA produced by transcription of w^{prx} and that the RNA acts as a corepressor by forming a complex with the zeste locus product. The close proximity of w^+ alleles simply increases the local concentration of the corepressor, thus increasing the local concentration of active repressor complex. The reaction can be summarized as

$$z^+ \text{ product} + w^{prx} \text{ RNA} \rightleftharpoons \text{active repressor.}$$

Mutations in w^{prx} affect both the ability of the white gene to produce pigmentation and its ability to activate zeste, while w^{dst} mutations affect only the former. This suggests that the white locus is polar in its organization. In prokaryotes such polarity is usually the result of having more than one cistron transcribed tandemly. One way to view the white locus is that it consists of two cistrons, but that the RNA specified by one of them, w^{prx}, is not translated. Rather, it is released during the processing of the initial white locus transcript and functions *in situ* as a component of a repressor complex.

The puff that Ashburner (10, 11) observed at 64C is particularly interesting in the context of a gene acting in its own regulation. The puff at 64C is observed in only one strain, *vg6*, among a number of *D. melanogaster* strains tested. As mentioned before, hybrids of *vg6* and Oregon-R show puffs on both chromosomes when the chromosomes are synapsed. However, the puffs in hybrids are reduced in size compared to *vg6* homozygotes. When the chromosomes are asynapsed in hybrids, the *vg6* chromosome has a normal-sized puff at 64C, while the homolog remains condensed. In hybrids of *vg6* with the sibling species *D. simulans*, both chromosomes fail to puff at 64C (11). Apparently, either the Oregon-R flies lack a 64C activator that

is provided by the *vg6* chromosomes or the *vg6* flies lack a repressor that is synthesized by the Oregon-R. Because the *vg6/simulans* hybrids are completely unpuffed at 64C and because *vg6*/Oregon-R hybrids have a reduced puff, the most likely explanation is that *vg6* flies are mutant for a repressor of 64C activity. Furthermore, the mutant causing puffing in *vg6* maps in the 64C area (11). Thus, the Oregon-R 64C gene is evidently active in its own repression.

The conclusion that a gene can participate in its own regulation appears warranted from the data we have presented. In our view the most logical of several explanations is that the regulation is mediated by an RNA transcribed by the gene being regulated. Regardless of the details of the mechanism of regulation, it appears inescapable that some aspects of the control of gene activity depend upon some type of communication between alleles. Our data show that this communication is strongly dependent on close proximity of alleles. This demands a rather precise nuclear architecture. From our results, we predict that in interphase nuclei, chromosomes occupy precise positions relative to one another and that an important aspect is the close association of homologs.

We especially thank Bill Gelbart for allowing us to use his extensive unpublished results and for his incisive comments on the manuscript.

We gratefully acknowledge helpful comments from George Lefevre and Larry Puckett and the valuable assistance of Margaret W. Shen. This study has been supported in part by Grant PCM-76-83056 from the National Science Foundation and the National Institutes of Health National Research Service Award 5T32 GM 07126 from the National Institute of General Medical Sciences.

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