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The *zeste-white* interaction: induction and genetic analysis of a novel class of *zeste* alleles

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The recovery and analysis of a novel class of *zeste* mutations is described. z^{op6} and z^{op11} do not require two w^+ genes for their expression unless the z^+ gene is present. Analysis of genetic interactions among *zeste* alleles proved that z^{op} mutations are strong antagonists of the z^+ gene product. z^{op6} is readily reverted by X-rays or by ethylmethane sulphonate to a range of intermediate *zeste* alleles and thus it is considered to be an insertional mutation. We suggest that z^1 and *op* mutations arose as a result of insertions in a presumptive control region, and two alternative models for the structure of the *zeste* locus are evaluated.

Key words: Drosophila/zeste alleles/*zeste-white* interaction

Introduction

Genetic mutations in presumptive regulatory elements are difficult to obtain, as most genetic systems in higher organisms are not sufficiently sensitive to changes in gene dosage. One such system is the well-known *zeste-white* interaction in *Drosophila melanogaster*. The *zeste* gene is located on the X-chromosomes, ~0.5 recombination units to the left of *white*, in close relation with band 3A3 (see Lindsley and Grell, 1968). The z^1 allele was identified as a recessive, lemon, eye color mutation, the expression of which was shown to depend on two doses of w^+ gene (Gans, 1953). Thus z^1w^+ females are zeste, but z^1w^+/z^1w^- are of wild-type eye color. Similarly, z^1w^+/Y males are normal, while an additional w^+ gene confers zeste phenotype in males as well. Green (1959) has shown that heterozygosity for mutations at the right hand site ($w^{proximal}$) of the *white* gene are sufficient to abolish the expression of homozygous z^1 in females, while Judd (1961) found that a tandem duplication of w^{prx} sites is as active as two w^+ doses. The w^{prx} sites (w^{ch}, w^{sp}) were suggested by Judd (1974) to define the regulatory elements of the *white* complex. Later, it was realised (Jack and Judd, 1979; Gelbart and Wu, 1982) that a possible transvection mechanism (Lewis, 1954) is involved, for the w^+ genes must be situated rather close to one another in order to allow expression of z^1. A seemingly unpaired w^+ gene is not suppressed by z^1.

The interpretation of the *z-w* interaction was inherently weak as it was assumed that the z^1 and z^a alleles adequately represent the range of mutations in this locus. The notion that '*w* is a suppressor of *z*' (Green, 1959; Judd, 1974) was challenged only in 1979. Reconsideration of long-existing data (Gans, 1953) on the genetic interactions of z^1 and z^a brought Jack and Judd (1979) to conclude, correctly, that z^+ and z^1 alleles are antagonistic.

Here we described experiments in which a novel class of *zeste* mutations was selected and analyzed. Unlike z^1, the z^{op} mutations confer a zeste phenotype in otherwise normal females, even if heterozygote with z^+ allele. Furthermore, when *op* mutations are homozygous, or when heterozygous with a *zeste* deletion, one dose of w^+ gene is sufficient for their phenotypic expression. Similarly, $z^{op}w^+/Y$ males, as well as $z^{op}w^+/Df(1)z\text{-}w$ females, are phenotypically perfect zeste. The most extreme *op* allele, z^{op6} reverts to weaker alleles at a frequency of 1×10^{-4} with ethylmethane sulphonate (EMS), or of 3×10^{-2} with X-rays.

As *op* mutations become useful for the molecular analysis of *zeste* and *z-w* interaction, we find it appropriate to present the formal description and genetic analysis as first drawn in 1977. In agreement with Jack and Judd (1979), we concluded that z^1, as well as z^{op6}, alleles actively antagonize the z^+ gene product. Furthermore, we believe that z^1 and *op* mutations arose as a result of insertions. The possibility that z^1 and *op* mutations activated an otherwise dormant *zeste*-type gene is discussed.

Results

A procedure for the recovery of zeste dominant mutations and white overproducing alleles

z^1w^+/z^1w^{ch} females are of wild-type eye color, as two w^{prx} genes are essential for the expression of z^1. If, however, a stronger *zeste* allele or overproducing mutations of w^{prx} are present, we might expect a zeste phenotype. Consequently, *sc* z^1 w^{ch} ec^+/sc z^1w^{ch} ec^+ females (w^{ch} in phenotype) were crossed with *sc* z^1 w^+ *ec*/*Y* males fed with 0.15% EMS. F1 z^1 w^{ch}/z^1 w^+ females are of normal color unless mutations that affect the expression of z^1 or w^+ genes occur. Among 52 000 F1 females, 13 exceptional females were found with eye color that ranged from brown to the typical lemon-yellow of zeste, with one instance of two brown-eyed females in the same bottle.

All zeste or brown-eyed females were crossed with their *sc* z^1 w^{ch}/Y brothers, after which five lines were discarded as autosomal enhancers. The remaining mutations were designated as *op* alleles of either locus. *op2, op5* and *op12* carried an additional lethal marker and were balanced over *FM6*. They proved to be *white* unstable duplications and were described in more detail by Green and Lefevre (1979). Together with *op7, op8* and *op9*, they were found to reside in or near the *white* gene. *op8* is a mutation to the right of w^a, but not in w^{prx}, which confers a zeste-like phenotype on *z* w^{op8}/Y males. *op7* and *op9* are also mutations in *white*, with no independent effect on eye color. However, *z* w^{op9}/Y males are zeste. The *op9* mutation segregates from *zeste* at the expected frequency, and *z* w^{op9}/Y males with zeste phenotype can be reconstructed at will. w^{op9} is a presumptive overproducer of w^{prx}. No further attention is paid in this article to w^{op} mutations.

Two *op* mutations induced in the *sc* z^1 w^+ *ec* chromosome were localized by recombination to a site 0.5 units distal to *white*. They did not interfere with recombination in the *y-w* region, and no z^1 segregants were found among >8000 F2

male progeny of *sc* z^{op6} *ec/y w sn* females (see Materials and methods). Furthermore, cytological examination of *op*-bearing chromosomes failed to reveal any recognizable change in the *z-w* region. The two alleles were given the notation z^{op6} and z^{op11}, and will subsequently be referred to as *op6* and *op11*. Both can be readily recombined with all *white* alleles to form doubly-marked chromosomes.

Genetic interactions of op6 and op11

op6 is 'dominant' to z^+, as *op6* w^+/z^+ w^+ gemales are zeste (combination A in Table I). w^{dis} alleles such as w^a (and 10 others) do not interfere with this reaction (Table I B, see Green, 1959). z^+, however, is 'dominant' to *op6* when only one w^{prx} gene is present as in *op6* w^+/z^+ w^- females (Table I E). *op6* is nevertheless expressed as a full zeste phenotype in the presence of one w^{prx} gene, provided that it is in a homozygous state or is heterozygous with either z^1 or a *zeste* deletion. *op6* w^+*/op6* w^-, *op6* w^-*/Df(z)* w^+ and *op6* w^+*/DF(z-w)* all exhibit a very bright zeste phenotype. Thus, under all circumstances, a deletion of z^+ enhances the expression of *op6, op11* or even z^1 alleles. An interesting, previously undescribed, phenomenon is observed when *op6/Df(z)* females bearing one or two w^+ genes are compared. *op6* w^+*/Df(z)* $w^+$are of typical zeste color, while *op6* w^+*/Df(z-w)* are bright zeste. It seems as though, in the absence of z^+, *op6* is somehow 'suppressed' by two doses of w^+. Although deletions of z^+ enhance the expression of the op phenotype, it is evident that *op* mutations themselves act in a manner opposite to *zeste* deletions. Thus, *Df(z)* w^+/z^+ w^+ is of wild-type eye color, but *op6* w^+/z^+ w^+ is zeste. Similarly, *Df(z)* w^+/z^1 w^{ch} females are of wild-type color, while *op6* w^+/z^1 w^{ch} are zeste. Additionally, *op6* cannot be a deletion because it mutates to other forms of *zeste* (see next section). Interestingly, unlike *Df(1)X10* in combination 1-P, *Df(1)*$w^{258\text{-}11}$ which is also supposed to be a *z-w* deletion (Kaufman *et al.*, 1975), exhibits a wild-type color when compounded with *op6* w^+. We presume, therefore, that a crucial z^+ element (see Discussion) is retained in the deleted chromosome. Thus, z^+ codes for an active product; *op6* elicits a zeste phenotype in a contrasting manner to that of a *zeste* deletion; and the activity of *op6* is enhanced by a deletion of z^+. *op6* thus also codes for an active product, but is not an overproducer of z^+. It is also impossible that z^1 is the hypomorph of z^+, as it affects eye color in the same way as *op6* does. The unavoidable conclusion is that *op6* and z^+ code for active antagonist products. The same conclusion was reached *vis à viz* z^1 and z^+ by Jack and Judd (1979), and it is not clear how it has escaped notice for so many years. A second important conclusion is that *op* mutations will elicit a zeste phenotype even in the presence of only one, unpaired, w^+ gene.

Since, unlike z^1, the expression of *op6* mutation does not depend exclusively on two doses of w^+, it is possible to compare the action of *zeste* in males and females under normal euploid conditions. *op6* w^+ homozygous females are bright-lemon color, while *op6* w^+/Y males manifest a darker zeste phenotype. *op6* w^+/z^+ w^+ females are lemon zeste, but *op6* $w^+/z^+w^+/Y$ males are brown. When *op11* and *RN4* (weaker derivatives of *op6*; see next section) are compared in the same way, it becomes clear that the more submissive to z^+ a *zeste* allele is, the greater is the difference in its phenotypic expression in males and females. Clearly, in addition to the doses of w^+ and the antagonistic capability of *op6*, the relative efficiency of the dosage compensation mechanism operating on the two genes is of prime importance. This is best demonstrated by the fact that *op6* w^+/Y males are dark zeste, but *op6* w^+*/Df(z-w)* females are bright zeste.

The other extreme *zeste* allele, *op11*, behaves in every respect as an intermediate between z^1 and *op6*. It is difficult to distinguish *op6* and *op11* in regular *op6* w^+/Y or *op11* w^+/Y males, as both give rise to the brown-zeste phenotype. The two alleles differ in their response in females (Table I G–J); *op11* w^+/z^+ w^+ females are of brown eye color, rather than zeste, and *op11* w^+/z w^{sp} are brown variegated, rather than brown-zeste, as in combination D (Table I). *op11* is therefore intermediate in its expression between z^1 and *op6*.

EMS-induced derivatives of z^{op6} *gene*

In order to determine the genetic nature of its dominance, reversions of *op6* were sought [the rationale of such experiments was outlined elsewhere (Lifschytz and Falk, 1969a; Lifschytz and Green, 1978)]. EMS-fed *sc op6* w^+ *ec/Y* males were crossed with *sc op6* w^- *sn* females. Regular F1 females are of the zeste phenotype (Table I F), overproducing mutations (or duplications) of w^{prx} are still zeste, while new *white* alleles confer the relevant white phenotype. Reversions of *op6* toward the wild-type state are expected to result in brown;variegated or wild-type eye color. Two brown-eyed females were found among 18 000 flies. Analysis of the isolated chromosomes (designated *RN1* and *RN4*) indicate that both are *zeste* alleles, and are localized by a recombination test to the *zeste* locus. *RN1* w^+/Y males are of red, variegated color (similar to w^{m4}/Y males), while *RN4* w^+/Y males are brown. From the interactions given in Table I Q–X, it is inferred that, in both cases, *op6* has been reverted to less extreme alleles, *RN1* being the least extreme. The hierarchy of the various *zeste* genes, in accordance with their effect on eye pigmentation is therefore *op6*>*op11*>*RN4* >z^1>*RN1*> wild type.

op mutations as possible insertions

In the stock, *sc op6* w^+ *ec/Y* and $\widehat{XX}/Y$, brown-eyed males were occasionally found. Three such exceptions proved to be inseparable from *zeste*. For example, z^{op6ld1} behaves exactly as does *RN4*. It is possible, therefore, that *RN1* and *RN4* themselves were not EMS-induced, but rather spontaneous changes in the state of the *op6* gene.

In a further search for *op6* revertants, *sc op6* w^+ *ec* males were irradiated with 4750 Rads and mated with *Basc* (M5)

Table I. Phenotypic expression of *zeste* alleles

A $z^{op6}w^+/z^+w^+$ = zeste	Q $z^{RN1}w^+/z^+w^+$ = wild-type (var.)
B $z^{op6}w^+/z^+w^a$ = zeste	R $z^{RN1}w^+/z^1w^+$ = brown (var.)
C $z^{op6}w^+/z^+w^{sp}$ = wild-type	S $z^{RN1}w^+/z^{op6}w^+$ = brown-zeste
D $z^{op6}w^+/z^1w^{sp}$ = brown	T Df(z) $w^+/z^{RN1}w^+$ = wild-type (var.)
E $z^{op6}w^+/z^+w^-$ = wild-type	U $z^{RN4}w^+/z^+w^+$ = brown
F $z^{op6}w^-/z^{op6}w^+$ = bright zeste	V $z^{RN4}w^+/zw^+$ = zeste
G $z^{op11}w^+/z^+w^+$ = brown	W $z^{RN4}w^+/z^{op6}w^+$ = zeste
H $z^{op11}w^+/z^+w^a$ = brown	X Df(z) $w^+/z^{RN4}w^+$ = brown
I $z^{op11}w^+/z^1w^{sp}$ = brown (var.)	
J $z^{op11}w^+/z^+w^-$ = wild-type	
K Df(z)*w^+/z^+w^+ = wild-type	
L Df(z) w^+/z w^+ = zeste	
M Df(z) $w^+/z^{op6}w^+$ = zeste	
N Df(z) $w^+z^{op11}w^+$ = zeste	
O Df(z) $w^+/z^{op6}w^-$ = bright zeste	
P Df(zw)*/$z^{op6}w^+$ = bright zeste	

*Df(z) is Df(1)X12, Df(zw) is Df(1)X10

homozygous females. Reversions of *op6* are easily recognizable, as regular F1 females are zeste, while newly induced *white* alleles are detected as the *Basc* chromosome carries the w^a allele. Of 40 748 F1 females, 141 (1/258) non-zeste daughters were found; all revertants were X-linked and none segregated as a dominant suppressor of z^{op6}. Twenty-nine chromosomes were of wild-type eye color when heterozygous with *Basc*, while the majority, 112, were of brown or brown-variegated eye color. Upon balancing and testing for interactions with *op6*, z^1 or *zeste* and *white* deletions, the newly-induced reversions were found to present the range of states between *op6* and *RN1*. None was z^+ and several (22) were associated with lethal effects, five of which were covered by w^+Y duplication. A frequency of 1/258, being too high, cannot be considered as a reversion rate of a point mutation, nor can the revertants themselves be the consequence of an induced point mutation.

Discussion

The *op* mutations were selected by screening for the expression of z^1 in the presence of one w^{prx} wild-type gene only. *op6* is more extreme than *op11* and induced partial revertants of *op6* further underscore the existence of a whole range of states in the *zeste* locus. The mere existence of *op*-like alleles proves, of course, that the somatic pairing of two w^+ genes is not important *per se*, and that the nature and dosage of *zeste* products are as crucial as the nature and dosage of the w^+ gene.

In the following analysis we shall discuss problems and possible genetic organizations for *zeste* only. Jack and Judd (1979) have demonstrated that the correct evaluation of long-existing data (Gans, 1953) on z^1 and z^a interactions favored the interpretation that z^+ and z^1 are antagonistic. Our analysis of the *op* mutations, is in agreement with this conclusion. The interpretation by Jack and Judd of the *z-w* interaction itself, however, is based on the assumptions the z^1 and z^a adequately represent the range of *zeste* alleles, and that the only role of w^{prx} sequences is to regulate the expression of the sole pigment-determining sites of w^{dis}. Consequently, their model was centered around the obligatory dependence of *z* expression on two paired w^+ genes. That *zeste* is represented by a much wider range of states is shown in this article and we believe that the arguments brought in favor of the simple two functional domain structured for *white* also deserve revision.

Models for the *zeste* locus must take into account the following facts: z^+ and *op* (or z^1) genes code for active products antagonistic to one another; mutated, antagonistic *zeste* alleles represent a wide range of gene states; *op* alleles of the *zeste* gene are unstable and easily mutated to intermediate states; the eye color phenotype is the consequence of a delicate balance between z^+ and z^{op} products on one hand and between the z^+/z^{op} product ratio and the w^{prx} gene product on the other. However, it does not depend exclusively or directly on any sort of physical interaction between the genes, (i.e., Jack and Judd, 1979).

Model A

z^1 and *op6* alleles are the consequence of neomorphic (or antimorphic) mutations of the z^+ structural gene itself. If accepted, this model requires that z^1 and *op* mutations not be the result of point mutations in the structural element of z^+ because *op6* was induced in a z^1 gene. If both were point mutations, *op6* would be double mutant, z^1z^{op6}, and accordingly *RN4* would be ($z^1z^{op6}z^{RN4}$) a triple mutant. Yet, it is difficult to see how the presumptive double mutant z^1z^{op6} is changed by an additional point mutation to the *RN4* state. The fact that both *op6* and *RN4* alleles are EMS-induced is immaterial because, as shown before (Lifschytz and Falk, 1969b), EMS also induces chromosomal aberrations. Most importantly, *op6* is reverted with exceptionally high frequency to intermediate states by X-rays, and thus cannot be considered as a point mutation. To adhere to a one-gene model, we must assume that *op6* (as well as z^1) are insertional mutations coding for new fusion proteins, which compete with the z^+ product for the, as yet evasive, interaction with the w^{prx} gene or its product. The different 'states' of *zeste* reflect the levels of antagonism to z^+ which is exerted by each of the many different fusion proteins. Based on this model, it is also possible that z^1 is an antagonistic mutation of the z^+ gene whose level of expression is increased by the *op* mutations.

Model B

Zeste is a complex locus composed of, at least, two related structural elements, z^+ and $z(H)^+$, the products of which antagonize each other. z^1 and *op* mutations condition the shift in $z^+/z(H)$ balance in favor of the *z(H)* gene. We speculate that the z^+ gene is a recent duplication of the ancestral *z(H)* gene. As a result of mutations (see Discussion by Ohno, 1970, 1973, 1974), the z^+ has acquired a base sequence more compatible with the currently favored eye color. Through the evolution of controlling elements (as studied by Ohno, 1974; Nei and Roychoudhury, 1973; Ferris and Whitt, 1977) the *z(H)* gene was fully or partially suppressed. Insertional mutations like z^1 and *op6* in the controlling element turned the clock back and resulted in different $z^+/z(H)$ product ratios. Model B best accounts for the antagonistic effect, for the paradox of dominance, for the instability and for the range of *zeste* states. It presumes that *op6* is in fact $z^+\ z(H)^{op6}$, and therefore does not exclude the synthesis of both antagonistic products in *op6* w^+ or z^1w^+ chromosomes. Formally at least, this model also allows for the differences in color intensities between *op6* $w^+/Df(z\text{-}w)$ females and *op6* w^+/Y males. If *z(H)* has evolved as a silent gene, its new active state may not conceivably abide by the rules of dosage compensation. The $z^+/z(H)$ ratio in the *op6* w^+/Y males will be in favor of z^+. It may also mean that w^{prx+} sites, like their mutated state, are not compensated in males. With certain additional, speculative assumptions the complex gene model can also explain the appearance of a *zeste* allele which, in contrast to z^1 and *op* alleles, is masked by two w^+ genes (Green, 1984).

Many facets of the *zeste-white* system will undoubtedly by solved by molecular analysis. As it turns out, genetics (and geneticists) failed to elucidate the underlying mechanism, probably because we were totally ignorant of the cellular level in which the *zeste* and *white* gene products operate and interact.

Materials and methods

D. melanogaster stocks

sc z w^+ *ec* homozygous females are zeste, but hemizygous males *sc z* w^+ *ec*/*Y* are of wild-type eye color.

sc z w^{ch} males and females are of w^{ch} phenotype. w^{ch} is a mutation at a w^{prx} site (Green, 1959), and consequently *sc z* w^{ch}/*sc z* w^+ females are wild-type. *Df(1)X10* and *Df(1)X12* were recovered as X-ray-induced lethals, covered by the w^+Y duplication (Lifschytz, 1967). *Df(1)X10* includes the *white* gene, while *Df(1)X12* does not. However, they were both shown to be deletions for the *zeste* gene (Kaufman *et al.*, 1975). *Df(1)X10* is therefore a z^-w^- marker while *Df(1)X12* is a z^-w^+ deletion. The z^+w^+Y chromosome is referred to in Lindsley and Grell (1968) as w^+Y. It contains, however, all wild-type genes

from *prune* to *Notch*, *zeste* included. Recombination experiments for the localization of the new *op* mutations were as follows. *y w sn* females were crossed to *sc z(op) ec/Y* males. F1 females were crossed with their *y w sn/Y* brothers and F2 males were scored for *y* w^+ or *sc w* recombinants. *y* w^+/Y males were tested for z^1 by crossing with *Df(1)X12/FM6* females (see text), while *sc w/Y* males were tested for the presence of z^1 or z^{op6} by crossing with *sc* z^{op6}*ec* females. *sc* z^+ w/Y males will give rise to wild-type F1 females. *sc* z^1 *w/Y* males will give rise to brown eye females while *sc* z^{op6} *w/Y* males will give rise to bright-zeste females. All other genetic markers, *white* alleles and balancer chromosomes are described by Lindsley and Grell (1968).

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