Heterochromatic *trans*-Inactivation of Drosophila *white* Transgenes

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ABSTRACT

Position effect variegation of most *Drosophila melanogaster* genes, including the *white* eye pigment gene, is recessive. We find that this is not always the case for *white* transgenes. Three examples are described in which a lesion causing variegation is capable of silencing the *white* transgene on the paired homologue (*trans*-inactivation). These examples include two different transgene constructs inserted at three distinct genomic locations. The lesions that cause variegation of *white* minimally disrupt the linear order of genes on the chromosomes, permitting close homologous pairing. At one of these sites, *trans*-inactivation has also been extended to include a vital gene in the vicinity of the *white* transgene insertion. These findings suggest that many Drosophila genes, in many positions in the genome, can sense the heterochromatic state of a paired homologue.

IN Drosophila, chromosomal rearrangements that juxtapose euchromatin and heterochromatin often cause variable silencing of reporter genes or position-effect variegation (PEV; see LOHE and HILLIKER 1995; WEILER and WAKIMOTO 1995; ELGIN 1996; HENIKOFF 1996 for recent reviews). For 70 years, PEV has aided in the investigation of heterochromatin, the regions of chromosomes that are rich in repeats, are poor in genes and are cytologically condensed during interphase. For instance, the study of dosage-sensitive modifiers of PEV has identified candidate genes that might be involved in the packaging of heterochromatin (GRIGLIATTI 1991). In addition, PEV has become a paradigm for epigenetic silencing phenomena in numerous organisms.

Among the most frequently studied gene reporters of PEV are *white* and *brown*, related genes that are reponsible for the deposition of pteridine pigments into the eye and other tissues of the fly. PEV of these genes is observed as mixtures of mutant and wild-type spots and patches of pigmentation scattered throughout the eye. *brown* is unusual compared to *white* and other genes that have variegating alleles in that PEV alleles of *brown* are dominant over wild-type alleles (GLASS 1933). Transcription of a wild-type *brown* allele is silenced by association with heterochromatin on the homologue, termed *trans*-inactivation (HENIKOFF and DREESEN 1989). *Trans*-inactivation depends on somatic pairing of homologous chromosomes during interphase (LIFSCHYTZ and HAREVEN 1982; KOPCZYNSKI and MUSKAVITCH 1992; HIRAOKA *et al.* 1993), and as such is one of several "*trans*-sensing" phenomena in Drosophila (TARTOF and HENIKOFF 1991). In contrast to dominant *brown* PEV alleles, all *white* PEV alleles are recessive. Why are these otherwise very similar genes so different with respect to silencing by heterochromatin in *trans*?

Here we show that *white* expression can also be silenced in *trans* by heterochromatin. This is remarkable in light of the fruitless attempts to find dominantly variegating *white* alleles (SPOFFORD 1976; J. SPOFFORD, personal communication). We describe three different examples in which dominant PEV of ectopic *white* alleles is observed. In all three, lesions leading to PEV do not involve gross chromosomal rearrangements and so are expected to minimally disrupt somatic pairing. This differs from the situation for the endogenous *white* gene, where chromosome pairing is grossly disrupted when *white* PEV alleles are heterozygous with $white^{+}$ (SPOFFORD 1976). We also demonstrate that a vital gene near an ectopic *white* insert can likewise be silenced in *trans* by heterochromatin. From these findings, we propose that many Drosophila genes can exhibit *trans*-inactivation. The reason *trans*-inactivation is seldom observed is that variegation-inducing rearrangements typically unpair homologues, preventing silencing in *trans*. *brown* may differ from *white* in that homologous association of the *brown* locus can remain intimate despite gross chromosomal rearrangements.

MATERIALS AND METHODS

Fly maintenance and stocks: Flies were maintained on cornmeal/molasses/agar medium in tubes at room temperature (22°) or 18°. Crosses were performed at 25°. In all stocks, the endogenous *white* gene was homozygous or hemizygous for a null mutation, $white^{1118}$.

The *white* transgenes described in this article include coding sequences and part of the flanking upstream region but lack identified enhancers. Figure 1A shows a comparison of

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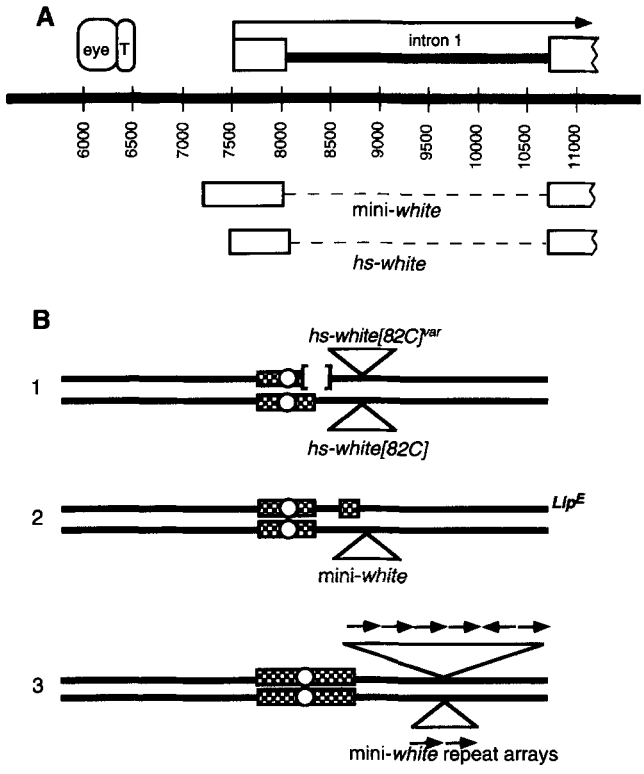

FIGURE 1.—(A) A genomic map of the endogenous *white* locus is shown, with coordinates based on GenBank sequence entry X02974. Above the line, the first exon and intron are diagrammed. Two enhancer regions, the eye enhancer and testis (T) enhancer, have been mapped (LEVIS *et al.* 1985) and are shown. Below, the 5′ *white* DNA that is included on the mini-*white* and *hs-white* transposons is indicated by open boxes. (B) Three examples of P[*white*$^+$] transposons that show dominant position effect variegation. (1) *hs-white* at 82C is *trans*-inactivated when heterozygous with any of four variegation-inducing lesions (indicated by open brackets). (2) Three separate mini-*white* transposon insertions at 83D are *trans*-inactivated by the *Lip*^{E} heterochromatic insertion into the same region. (3) A duplication of mini-*white* at 50C is *trans*-inactivated by transposon repeat arrays at the same site. Checkerboard blocks, location of heterochromatin; circles, centromeres.

the endogenous *white* gene and the transgenes used in this study. P[*hs-white*] at 82C (RS3-3) and an unlinked *hs-white* transposon insert (RS3-2) were obtained from K. GOLIC (GOLIC and GOLIC 1996). Both contain the *hsp70* promoter, *flp* recombinase targets and the *white* gene (Figure 1A shows the 5′ extent of *white* included on this construct). P[*lacw*] (mini-*white*) at 50C was generated by transposition and amplification of a two-copy array of mini-*white* from 92E (DORER and HENIKOFF 1994). Three P[*lacw*] insertions at 83D were obtained from the Berkeley Drosophila Genome Project (http://fruitfly.berkeley.edu/). mini-*white* at 61E-"40B6" has been previously described (WINES and HENIKOFF 1992).

The variegating rearrangements of the 83D, 50C and 61E mini-*white* transgenes have been described by CSINK *et al.* (1994), DORER and HENIKOFF (1997) and WINES *et al.* (1996), respectively. Figure 1B depicts the 83D and 50C transgene insertions and heterochromatic rearrangements that cause these *white* transgenes to variegate. P[99B]Δ2,3 contains a stable source of genomically encoded transposase, which functions in somatic as well as germline tissues (ROBERTSON *et al.* 1988). In some crosses, P[99B]Δ2,3 was carried on a third chromosome marked with *Stubble* (*Sb*).

Su(var)2^{01}, *Su(var)205* and *Su(var)208* mutant lines were obtained from G. REUTER. To test the effect of a *Su(var)* on the eye phenotype and viability of different variegating alleles, each *Su(var)* was balanced with a chromosome bearing a dominant phenotypic marker. *Su(var)*$^-$ flies were those that did not express the dominant phenotype; conversely, their balancer-carrying siblings were designated *Su(var)*$^+$.

Df(3R)110 and *Df(3R)Z1* were obtained from the Bloomington Stock Center. In tests of the viability of flies carrying these deficiencies heterozygous with a *hs-white* insert at 82C, *Df(3R)110/TM3* or *Df(3R)Z1/MKRS* were mated with *hs-white* homozygotes. The viability of deficiency/*hs-white* flies was compared with the viability of their balancer-carrying siblings.

Mutagenesis: X-ray mutagenesis was performed to obtain variegating alleles of *hs-white*. Homozygous *hs-white* males were exposed to 3000 rad and mated with w^{1118} females. Four variegating progeny were obtained and mated again to w^{1118}. Each allele proved to be homozygous lethal and so was kept balanced with *TM3*. Figure 1B depicts the lesions that cause variegation of *hs-white* at 82C.

P-element excision mutagenesis: The P[99B]Δ2,3 transposon was used to activate mobility and excision of the *hs-white* transposon at 82C. P[99B]Δ2,3/ *hs-white* heterozygotes were mated to w^{1118}; *TM6B/TM3* females and *white*$^-$ balanced progeny were selected. These were mated to w^{1118}; *TM6B/TM3* so that *hs-white*^{excised}/balancer flies could be bred.

RESULTS

A *hs-white* transgene shows dominant PEV: Flies bearing a transposon with *hsp70-white* (*hs-white*; see Figure 1A) inserted at 82C have uniform pigmentation. Four different variegating alleles of this *hs-white* transgene (*hs-white*[*82C*]) were generated by X-ray mutagenesis (Figure 2, A–D). Three of the lesions that result in PEV of *hs-white*[*82C*] are undetectable in salivary gland polytene chromosomes (data not shown). Owing to the extreme proximal location of the transgene insertion, rearrangement breakpoints within or immediately adjacent to centric heterochromatin would be difficult to detect. Nevertheless, in one variegating allele (*hs-white*[*82C*]^{var3}), there is some distortion of the banding in the interval proximal to the transposon, suggestive of a small neighboring chromosomal deletion that brings heterochromatin (checkerboard box in Figure 1B) closer to the 82C insert.

One might expect that heterozygotes of a variegating allele and its nonvariegating parental allele would have an additive amount of pigment, or at least the pigment level associated with the parental allele. However, we find that such heterozygotes have eyes with patches of unpigmented eye tissue, indicating that the parental *hs-white* transgene is being *trans*-inactivated. The extent of *trans*-inactivation is correlated with the extent of *cis*-inactivation, as shown in Figure 2, A-D and diagrammed in Figure 3. The *hs-white*[*82C*]^{var3} derivative has no effect on expression of *hs-white* transposons located elsewhere in the genome, suggesting that *trans*-inactivation requires pairing of homologues (Figure 2E).

The *hs-white* gene in *cis* to the heterochromatic lesion

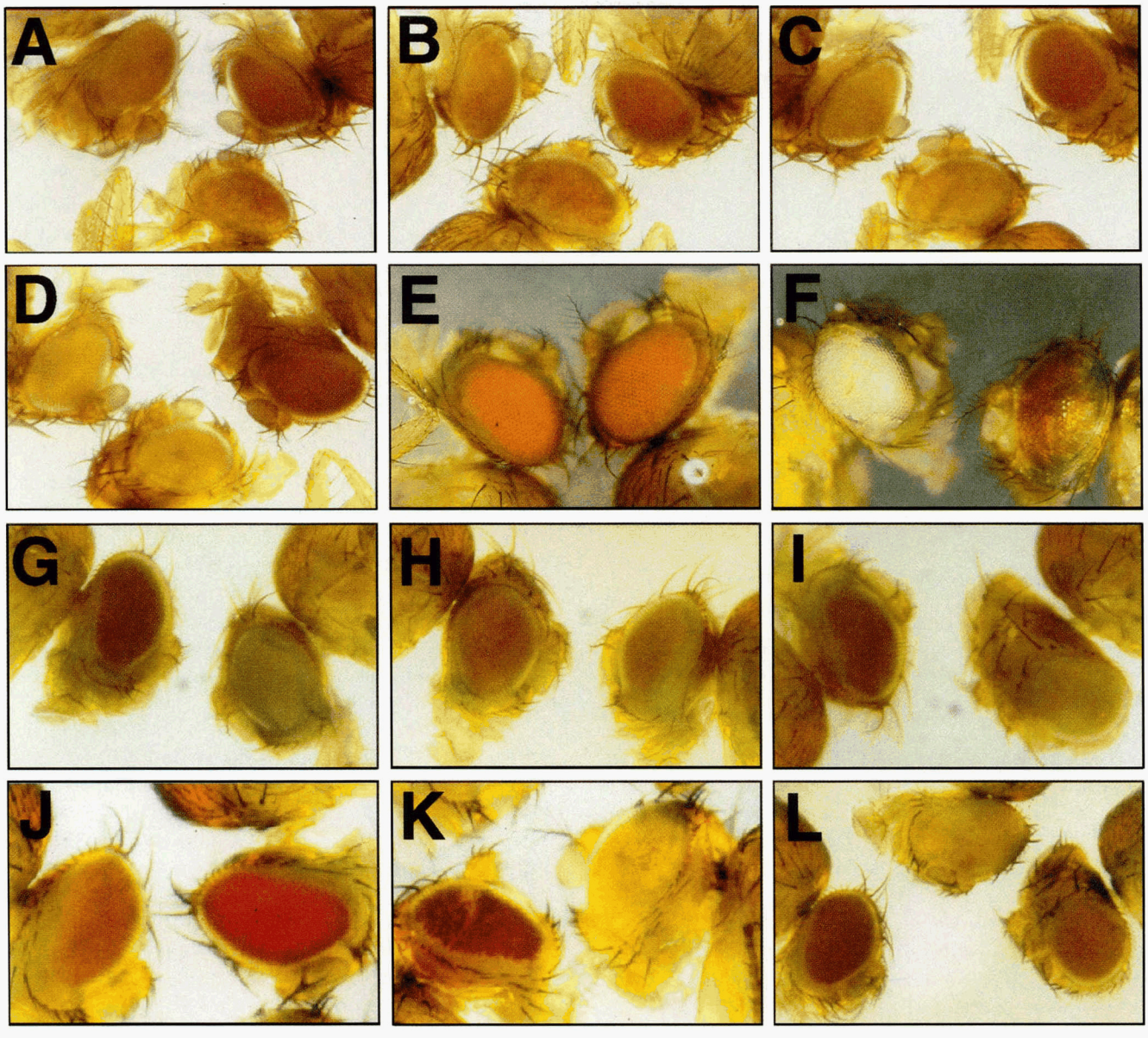

FIGURE 2.— (A–D) Flies bearing *hs-white* and *hs-white*^{var} allelic combinations. In each case, hemizygous *hs-white* is on the right, hemizygous *hs-white*^{var} is on the left and *hs-white*[*82C*]^{var}/*hs-white*[*82C*] is at the bottom. (A) *hs-white*[*82C*]^{var1}/*hs-white*[*82C*], (B) *hs-white*[*82C*]$^{var1'}$, (C) *hs-white*[*82C*]^{var2} and (D) *hs-white*[*82C*]^{var3}. In all *hs-white*[*82C*]^{var}/*hs-white*[*82C*] eyes, unpigmented patches are observed. (E) *hs-white*[*82C*]^{var3} on chromosome *3* does not affect a *hs-white* insert located elsewhere in the genome. *hs-white[82C]*^{var3}*/+; RS3-2/+* (left) and *RS3-2/RS3-2* (right) flies are shown. No unpigmented tissue is detected. (F) *hs-white*[*82C*]^{var3}/*hs-white*[*82C*] sibling flies that are either suppressed by *Su(var)208* (right) or not (left). Suppression was also seen with *Su(var)2*^{01} and *Su(var)205*. (G–I) Flies bearing various mini-*white* *P*[*lacw*] insertions in the 83D region heterozygous with either a balancer chromosome *(TM3, Ser)* (left of each frame) or *Lip*^{E} (right). Stock designations for P insertion lines are from the Berkeley Drosophila Genome Project: (G) j3D2 (H) 14E8 and (I) j3D5. (J and K) Flies carrying mini-*white* insertions from *P*[*lacw*] at 50C are shown. (J) Hemizygous (left) and homozygous (right) flies from a one-copy line show that doubling the copy number results in greater pigment in the eye. (K) Hemizygous (left) and homozygous (right) flies from a six-copy line show that a homozygote has far fewer pigmented ommatidia than a hemizygote for the array. (L) A mini-*white* transgene that does not show *trans*-inactivation by a variegating rearrangement. A fly hemizygous for the 40B6 mini-*white* insertion at 61E is shown on the left. In the middle is a fly hemizygous for the heterochromatic rearrangement *T(2;3)Ch* that causes this transgene to variegate. On the right, an eye from a 40B6/*T(2;3)Ch* heterozygote displays no unpigmented patches.

was mutagenized using transposase encoded by P[99B]Δ2,3. Examination of 10 independent *white*$^{-}$ derivatives, presumed to result from the excision of all or part of *hs-white,* revealed patches of unpigmented tissue in *hs-white*[*82C*]*/hs-white*[*82C*]$^{var\text{-}excised}$ flies. This demonstrates that the excision alleles can still *trans*-inactivate the parental *hs-white* allele. This situation is similar to that for *brown* (GLASS 1933; DREESEN *et al.* 1991), where the *cis* allele is not required for *trans*-inactivation of *brown*$^{+}$ on the homologue. *trans*-inactivation of *hs-white*

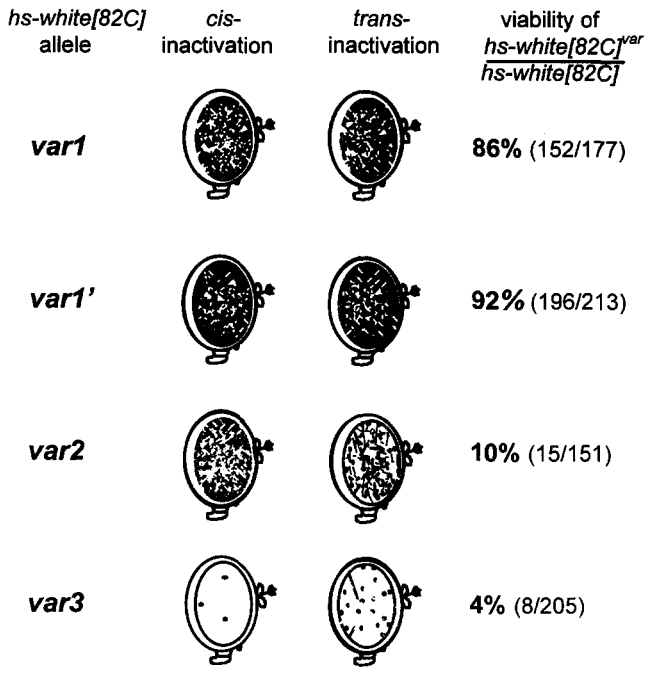

FIGURE 3.—Correlation between PEV silencing of *white* and lethality for four *hs-white*[*82C*]^{var} alleles. The four different *hs-white* [*82C*]^{var} are depicted as hemizygotes and as heterozygotes with *hs-white*[*82C*]. The severity of gene silencing in *cis* as well as the severity of *trans*-inactivation is diagrammed. The extent of lethality associated with the *hs-white*[*82C*]^{var}/*hs-white*[*82C*] genotype is also shown for each allele, where numbers in parentheses denote the ratio of *hs-white*[*82C*]^{var}/*hs-white* [*82C*] to balancer/*hswhite*[*82C*] siblings. Note that the most extreme allele, *hs-white*[*82C*]^{var3}, shows most gene silencing in *cis* and *trans* and is also associated with the highest degree of lethality.

is suppressed by *Suppressors-of-variegation, [Su(var)s]*, which act generally to suppress PEV mutations (GRIGLIATTI 1991). Figure 2F shows the effect of *Su(var)205* on the *trans*-inactivation induced by the most severe allele *hs-white*[*82C*]^{var3}.

mini-*white* transgenes can be *trans*-inactivated: It was important to determine whether sensitivity to heterochromatin in *trans* could be generalized to other *white* transgenes (see Figure 1A for a comparison of *white, hs-white* and mini-*white*). We examined three insertions of the mini-*white* transgene within the 83D region, a proximal location that is ~10% of the distance from the pericentric heterochromatin of 3R to the telomere. These transposon insertions are alleles of the *Lighten-up* (*Lip*) gene (A. K. CSINK, unpublished results). Another allele, Lip^E, is associated with a heterochromatic insertion at 83D (CSINK *et al.* 1994). When these mini-*white* insertion alleles are heterozygous with Lip^E, they are *trans*-inactivated (Figure 2, G–I). Lip^E does not affect the expression of single mini-*white* insertions at either 50C or 92E, indicating that homologous pairing is required for *trans*-inactivation and that *Lip* does not regulate mini-*white*. The degree of *trans*-inactivation caused by Lip^E is variable depending on the location of the mini-*white* insertion, which is remarkable considering that the insertion sites of mini-*white* at 83D have been mapped to within 400 base pairs of each other (D. HEKMAT-SCAFE, personal communication). We have not excluded the possibility that genetic background differences account for this variability, although all three lines are from the same collection.

Another site at which mini-*white* is sensitive to *trans*-inactivation is 50C, where arrays of mini-*white*-bearing transposons have been shown to exhibit properties of heterochromatin (DORER and HENIKOFF 1994). The variegated phenotype associated with a six-copy array is dominant over the unvariegated phenotype of a two-copy array at the same site (DORER and HENIKOFF 1997). Arrays at 50C have no effect on mini-*white* transposons at other sites. A *trans* interaction between arrays is implicit from the observation that homozygotes for a six-copy array have less-pigmented eyes than the corresponding six-copy hemizygotes (Figure 2K). No comparable *trans* interaction is seen for one-copy homozygotes, which have more pigmented eyes than one-copy hemizygotes (Figure 2J). The former result is reminiscent of the observation that classical variegating rearrangements, such as *white*^{mottled4}, result in more extreme silencing of *white* as homozygotes than as heterozygotes over a *white null* allele (SPOFFORD 1976). We have recently confirmed this observation both with *white*^{mottled4} and *white*$^{mottled\ McLean}$ (data not shown).

Whereas we have described three situations in which *white* transgenes can be *trans*-inactivated, this is not the case for all *white* transgenes. A transposon carrying the mini-*white* gene located at 61E (designated 40B6) was also examined. A translocation [*T(2;3)Ch, 2het;61E*] of the chromosome carrying this insertion causes moderately strong PEV of the mini-*white* gene (WINES *et al.* 1996). 40B6/*T(2;3)Ch* flies have uniformly pigmented eyes and show no evidence for *trans*-inactivation of *white* at 61E (Figure 2L).

***trans*-inactivation of a nearby vital gene:** The heterochromatic lesions at 82C that cause *hs-white* to variegate (Figure 1B, 1) also affect viability. Flies that are hemizygous or homozygous for the *hs-white*[*82C*] (Figure 4, A and B) are fully viable. Mutants carrying any of the four lesions that cause this insert to variegate are lethal as homozygotes (Figure 4D). When each *hs-white*[*82C*]^{var}/balancer line was crossed to a homozygous stock carrying the unrearranged *hs-white* insert, fewer *hs-white*[*82C*]^{var}/*hs-white*[*82C*] flies were observed than expected (Figure 4E). Figure 3 shows the percentage of flies of the *hs-white*[*82C*]^{var}/*hs-white*[*82C*] genotype for each variegating allele of *hs-white* compared to expectation based on frequency of the *hs-white*[*82C*]/balancer class. Comparing the weakest variegator (*hs-white*[*82C*]$^{var1'}$) with the most extreme variegator (*hs-white*[*82C*]^{var3}), the extent of lethality directly parallels the extent of silencing, both in *cis* and *trans*.

Does heterochromatin by itself cause *trans*-inactivation

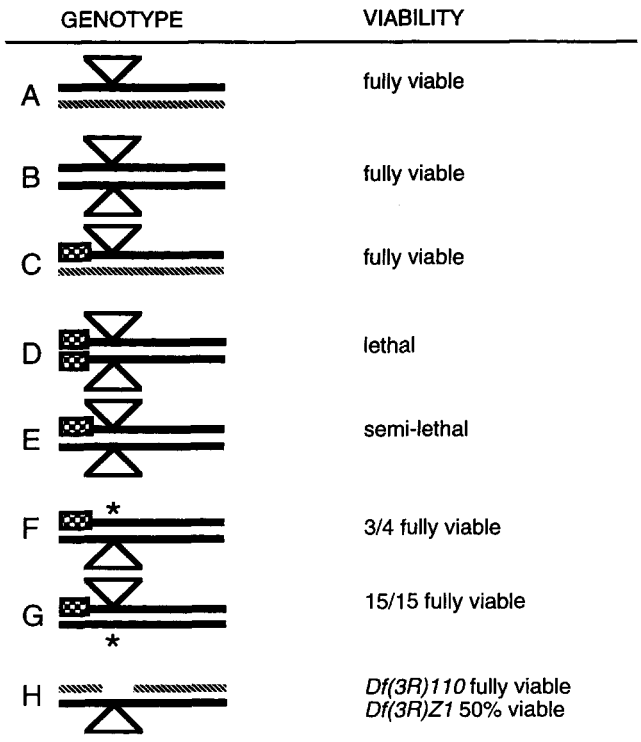

FIGURE 4.—Diagrammatic genotypes of various combinations of *hs-white* alleles and their viability. The *hs-white*[*82C*] insertion is viable as a hemizygote (A) and homozygote (B). The solid bar represents the parental third chromosome onto which the *hs-white* transgene was inserted. The stippled bar indicates an unrelated third chromosome. *hs-white*[*82C*]^{var} derivatives are viable as hemizygotes (C) but lethal as homozygotes (D). (E) *hs-white*[*82C*]^{var}/*hs-white*[*82C*] flies survive at reduced frequencies compared to their *hs-white*[*82C*]^{var}/balancer siblings. The extent of lethality is correlated with gene silencing (see Figure 3). Transposase-induced deletion (*) of the *hs-white* transposon located on the rearranged (F) or parental (G) chromosome can ameliorate the lethality associated with the *hs-white*[*82C*]^{var}/*hs-white*[*82C*] genotype. Full viability was obtained using *hs-white*[*82C*]$^{var3\text{-}null}$/*hs-white*[*82C*] in F and *hs-white*[*82C*]^{var3}/*hs-white*[*82C*]^{null} in G. (H) Two different deletions that remove sequences surrounding 82C were tested in flies carrying the *hs-white*[*82C*] transposon. Flies of these genotypes were viable.

of a vital gene near 82C or are the transposon insertions also required? To distinguish these possibilities, we asked if removal of the transposon on the chromosomes in question would reduce or ameliorate the lethality associated with the *hs-white*[*82C*]^{var}/*hs-white*[*82C*] genotype (Figure 4, F–H). Four *white*$^-$ null derivatives were obtained by P-element mobilization and tested for viability in the *hs-white*[*82C*]$^{var\text{-}null}$/*hs-white*[*82C*] genotype. Three of the lines in which the transposon located in *cis* to the heterochromatic lesion was excised display full viability of the *hs-white*[*82C*]$^{var\text{-}null}$/*hs-white*[*82C*] genotype (Figure 4F). It is possible that differences between *white*$^-$ excision lines are due to differences in deleted material, as it has been established in other studies that P-transposase-induced deletions typically have variable breakpoints both inside and outside the transposon (DREESEN *et al.* 1991; DANIELS and CHOVNICK 1993). In all 15 lines in which the transposon in *trans* to the heterochromatic lesion was null for *white*, the viability of the *hs-white*[*82C*]^{var}/*hs-white*[*82C*]^{null} genotype was fully restored (Figure 4G). The restoration of viability with manipulation of the transposon in *cis* or *trans* demonstrates the requirement for the transposon in *trans*-inactivation of the vital gene(s).

Is heterochromatic silencing of a vital gene responsible for the lethality associated with *hs-white*[*82C*]^{var} chromosomes? Escapers of the *hs-white*[*82C*]^{var2}/*hs-white*[*82C*] and *hs-white*[*82C*]^{var3}/*hs-white*[*82C*] genotypes were smaller and emerged with the desiccated appearance of aged flies. Presumably, the abnormal appearance of escapers results from leaky expression of a variegating vital gene or genes. We tested the effects of *Su(var)s* on this escaper phenotype and found that both *Su(var)2*^{01} and *Su(var)205* restore the normal appearance of escapers. The 14 *Su(var)*$^-$ escapers of the *hs-white*[*82C*]^{var2}/*hs-white*[*82C*] genotype were all of normal appearance, in contrast to their seven *Su(var)*$^+$ siblings, which were abnormal [χ^2 = 21 (1 d.f.); $P \ll 0.001$]. Likewise, the four *Su(var)*$^-$ flies of the *hs-white*[*82C*]^{var3}/*hs-white*[*82C*] genotype were normal, unlike their single severely affected *Su(var)*$^+$ sister. We conclude that heterochromatin causes variegation of a vital gene(s), and that genes other than *brown* and *white* can be silenced by heterochromatin in *trans*.

Lethality of *hs-white*[*82C*]^{var}/*hs-white*[*82C*] flies might be attributed to combined repressive effects of the transposon and heterochromatin on the expression of a vital gene (Figure 5A). To test this possibility, we examined the viability of flies carrying one of two different deletions of the 82C region on one chromosome and *hs-white*[*82C*] on the homologue (Figure 4H). If lethality results from the combined effects of transposon insertion and heterochromatin in *hs-white*[*82C*]^{var3}/*hs-white*[*82C*], then we would expect that a deficiency of this vital gene would be more detrimental to gene activity than *hs-white*[*82C*]^{var3}. *Df(3R)110* is deficient for 82C4-82F3/7. Flies heterozygous for this deficiency and *hs-white*[*82C*] eclosed at the same frequency as their *hs-white*[*82C*]/balancer siblings. These flies are not phenotypically different from siblings. *Df(3R)Z1* is deficient for 82A5/6-82E4. *hs-white*[*82C*]/*Df(3R)Z1* flies were half as numerous as their *hs-white*[*82C*]/balancer siblings, and they did not have noticeable phenotypic abnormalities. A chromosome carrying a deficiency of the entire region has much less severe consequences than a chromosome with *hs-white*[*82C*]^{var3} on the phenotype of flies that also carry *hs-white*[*82C*]. Therefore, we conclude that heterochromatin silences the vital gene on both homologues (Figure 5B).

DISCUSSION

We have described three chromosomal sites at which inserted *white*-bearing transposons are associated with

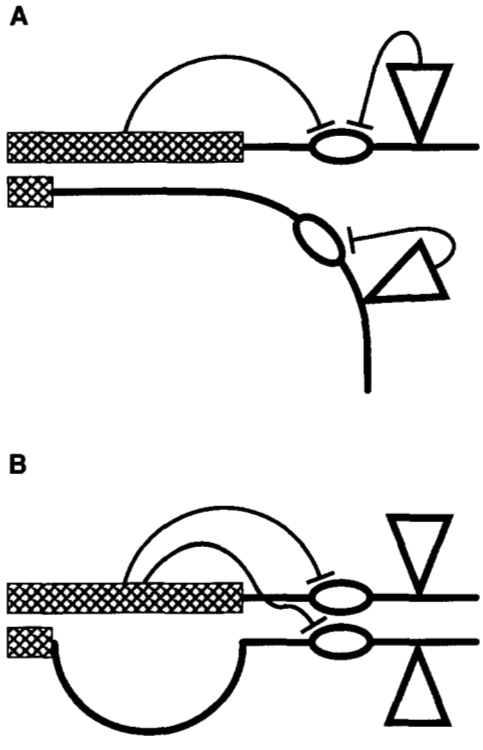

FIGURE 5.—Alternative models explaining the lethality of the *hs-white*[*82C*]^{var}/*hs-white*[*82C*] genotype. (A) A vital gene (oval) is proposed to reside near to the *hs-white* insertion at 82C. The expression of this gene would be affected by the transposon insertion (▽) as well as the juxtaposition of heterochromatin (crosshatched). Each effect would be independent, and only when this gene has suffered from three "strikes" is expression reduced below a threshold of viability. (B) Heterochromatin is proposed to be capable of silencing a vital gene, in *trans* as well as in *cis*. Heterochromatin-induced *trans*-inactivation is suppressed by local distortions of pairing, such as the removal of the transposon on either homologue.

heterochromatin-induced *trans*-inactivation. The role of heterochromatin in *trans*-inactivating *white* transgenes and a vital gene at 82C is confirmed by the observation of suppression by *Su(var)* mutations. Sensitivity to *trans*-inactivation is not restricted to any promoter type, as susceptible genes carry tissue-specific (*brown*), basal (mini-*white*) and inducible (*hs-white*) promoters. Moreover, high levels of transcription do not overcome silencing by *trans*-inactivation, because *trans*-inactivation occurs to the same extent whether or not *hs-white* is induced during development (L. MARTIN-MORRIS, unpublished results).

Our detection of *trans*-inactivation of *white* transgenes raises the question of whether endogenous *white* is also sensitive, but too weakly to be detected. In support of this interpretation, we note that, under special circumstances, *white* becomes sensitive to the heterochromatic state of its homologue. *white*^{is} flies are phenotypically wild type, but unlike *white*$^{+}$, *white*^{is} is repressed in *zeste*^{1} males (RASMUSON-LESTANDER *et al.* 1993). The inversion chromosome *In(1)w*^{is} is a variegating derivative of the chromosome bearing the *white*^{is} allele. *In(1)w*^{is}/*white*$^{+}$ flies are solidly pigmented but, in a *zeste*^{1} *background*, *In(1)w*^{is}/*white*$^{+}$ flies display a variegating phenotype. It is interesting that *In(1)w*^{is}/*white*$^{+}$ requires a mutation of *zeste* to show *trans*-inactivation. *zeste* mutations are known to influence other genotypes that are sensitive to the pairing state of homologous chromosomes (JUDD 1988). *zeste*^{1} encodes a protein that has been proposed to help paired chromosomes cohere more tightly (BICKEL and PIRROTTA 1990). In the presence of *zeste*^{1}, closer association of *white*$^{+}$ and *In(1)w*^{is} may explain the sensitivity of *white*$^{+}$ to the heterochromatic state of *In(1)w*^{is}.

Other evidence that the chromosomal *white* gene can be made sensitive to silencing by heterochromatin in *trans* derives from examination of *white*^{mottled} homozygotes. It had been noticed that *white*^{mottled} homozygotes are less pigmented than would be anticipated if each *white* allele were able to make independent pigment contributions (SPOFFORD 1976), and we have confirmed this for both *white*^{mottled4} and *white*$^{mottled\ McLean}$. Each variegating allele appears capable of interacting in *trans* with the other allele in a homozygote, causing some degree of silencing on the homologue. These results suggest that endogenous *white* can indeed show *trans*-silencing.

We have described several examples in which *trans*-inactivation is observed for *white* transgenes and one case where it is not. For each example in which ectopic *white* is *trans*-inactivated, the variegation-inducing lesion is minimally disruptive, preserving the linear arrangement of genes on the chromosome and permitting near-normal pairing (Figure 1B). For *hs-white*[*82C*]^{var}, the 82C region appears normally paired in polytene chromosomes, with the exception of *hs-white*[*82C*]^{var3}/*hs-white*[*82C*], which causes only a minor distortion. *Lip*^{1} is an insertion of heterochromatin near 83D that leaves the surrounding chromosome sequence undisturbed, permitting normal pairing of flanking regions. The 50C mini-*white* repeat arrays are even smaller insertions, minimally disrupting the pairing of flanking regions. No pairing disruption occurs at all in *white*^{mottled4} and *white*$^{mottled\ McLean}$ homozygotes. In contrast, mini-*white* at 61E cannot be *trans*-inactivated by *T(2;3)2het;61E*. This rearrangement grossly alters the chromosome configuration, presumably leading to unpairing. We conclude that *trans*-inactivation of *white* transgenes requires close apposition of homologues. This model might account for the restoration of viability observed for *white*$^{-}$ derivatives of *hs-white*[*82C*]^{var}/*hs-white*[*82C*]: suppression of *trans*-inactivation might reflect unpairing caused by loss of *white*.

The importance of close pairing for *trans*-inactivation had been previously speculated to underlie the unusual strength of *trans*-inactivation by the *brown*^{Dominant} heterochromatic insertion (HENIKOFF and DREESEN 1989). At that time it appeared that the *brown* gene was highly unusual in being silenced by heterochromatin in *trans*. However, our detection of *trans*-inactivation of *white* and

a vital gene(s) at 82C suggests that genes differ only in their sensitivity to pairing disruptions. *brown* may be so insensitive that *trans*-inactivation occurs even when chromosomal pairing is apparently disrupted by a gross rearrangement. Pairing would be so intimate that rearrangements involving *brown* do not result in unpairing of *brown* alleles. Nuclear compartmentalization appears to underlie *trans*-inactivation of *brown* (TALBERT *et al.* 1994; HENIKOFF *et al.* 1995; CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996), and recent genetic results have extended this interpretation to *trans*-inactivation of mini-*white* repeat arrays at 50C (DORER and HENIKOFF 1997). Homologue "dragging" of a susceptible reporter gene into a heterochromatic compartment of the nucleus (HENIKOFF *et al.* 1995) might be the basis for *trans*-inactivation in general.

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