Heterochromatic trans-Inactivation of Drosophila white Transgenes

Linda E. Martin-Morris, Amy K. Csink, Douglas R. Dorer, Paul B. Talbert and Steven Henikoff

Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109-1024

Manuscript received March 24, 1997
Accepted for publication June 25, 1997

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 responsible 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 (LIFSCHTZ and HAREVEN 1982; KOPCZYNKI 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 wild-type (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°C). Crosses were performed at 25°C. In all stocks, the endogenous white gene was homozygous or hemizygous for a null mutation, whitea.

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
the endogenous *white* gene and the transgenes used in this study. *P*[hs-*white*] at 82C (RS3-5) and an unlinked *hs-white* transposon insert (RS3-2) were obtained from K. Golic (Golic and Golic 1996). Both contain the *hs70* 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 (Dorér and Henikoff 1994). Three *P*[lacw] insertions at 83D are trans-inactivated by the *L(p)het* heterochromatic insert 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.

**FIGURE 1.**—(A) A genomic map of the endogenous *white* locus is shown, with coordinates based on GenBank sequence entry X92974. 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* heterochromatic insert 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.

In some crosses, *P*[99B]*Δ2.3* was carried on a third chromosome marked with *Stubble* (Sb). *Su(var)205*, *Su(var)205* and *Su(var)208* mutant lines were obtained from C. 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)*

**RESULTS**

A *hs-white* transgene shows dominant PEV: Flies bearing a transposon with *hs70-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]*)*, 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]*Δ2.3* 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" allelic combinations. In each case, hemizygous hs-white is on the right, hemizygous hs-white" is on the left and hs-white[82C]"/hs-white[82C] is at the bottom. (A) hs-white[82C]"/hs-white[82C], (B) hs-white[82C]"/hs-white[82C], (C) hs-white[82C]" and (D) hs-white[82C]". In all hs-white[82C]"/hs-white[82C] eyes, unpigmented patches are observed. (E) hs-white[82C]" on chromosome 3 does not affect a hs-white insert located elsewhere in the genome. hs-white[82C]"/+; RS3-2/+ (left) and RS3-2/RS3-2 (right) flies are shown. No unpigmented tissue is detected. (F) hs-white[82C]"/hs-white[82C] sibling flies that are either suppressed by Su(var)208 (right) or not (left). Suppression was also seen with Su(var)208 and Su(var)205. (G–I) Flies bearing various mini-white P{lac} insertions in the 83D region heterozygous with either a balancer chromosome (TM3, Ser) (left of each frame) or Lif (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{lac} 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]" 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 1953; DREESEN et al. 1991), where the cis allele is not required for trans-inactivation of brown" on the homologue. trans-inactivation of hs-white...
which act generally to suppress mal location that is -10% of the distance from the chromatin in trans could be generalized to other white alleles. These transposon insertions are alleles of the Lighten-up (Lip) gene (A. K. CSINK, unpublished results). 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\textsuperscript{male}\textsuperscript{4}, 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\textsuperscript{male}\textsuperscript{4} and white\textsuperscript{male}\textsuperscript{Melon} (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 [\textit{T}(2;3)Ch, \textit{2het}; 61E] of the chromosome carrying this insertion causes moderately strong PEV of the mini-white gene (WINES et al. 1996). 40B6/\textit{T}(2;3)Ch flies have uniformly pigmented eyes and show no evidence for trans-inactivation of white at 61E (Figure 2L).

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

Does heterochromatin by itself cause trans-inactivation

---

**Figure 3.**—Correlation between PEV silencing of \textit{white} and lethality for four \textit{hs-white}[82C]\textsuperscript{var} alleles. The four different \textit{hs-white}[82C]\textsuperscript{var} are depicted as hemizygotes and as heterozygotes with \textit{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 \textit{hs-white}[82C]\textsuperscript{var}/\textit{hs-white}[82C] genotype is also shown for each allele, where numbers in parentheses denote the ratio of \textit{hs-white}[82C]\textsuperscript{var}/\textit{hs-white}[82C] to balancer/\textit{hs-white}[82C] siblings. Note that the most extreme allele, \textit{hs-white}[82C]\textsuperscript{var}, shows most gene silencing in cis and trans and is also associated with the highest degree of lethality.
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 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], then we would expect that a deficiency of this vital gene would be more detrimental to gene activity than hs-white[82C]/hs-white[82C]. 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]/ 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
heterochromatin-induced trans-inactivation. The role of heterochromatin in trans-inactivating white transgenes and a vial 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 flies are phenotypically wild type, but unlike white, white is repressed in zeste males (Rasmuson-Lestander et al. 1993). The inversion chromosome In(1)wa/white is a variegating derivative of the chromosome bearing the white allele. In(1)wa/white flies are solidly pigmented but, in a zeste background, In(1)wa/white flies display a variegating phenotype. It is interesting that In(1)wa/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 encodes a protein that has been proposed to help paired chromosomes cohere more tightly (Bickel and Pirrotta 1990). In the presence of zeste, closer association of white and In(1)wa may explain the sensitivity of white to the heterochromatic state of In(1)wa.

Other evidence that the chromosomal white gene can be made sensitive to silencing by heterochromatin in trans derives from examination of white homozygotes. It had been noticed that white 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 and white Mclean. Each variegating allele appears capable of interacting in trans with the other allele in a homogygote, 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] and the 82C region appears normally paired in polytene chromosomes, with the exception of hs-white[82C] and hs-white[82C], which causes only a minor distortion. Lpf 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 and white Mclean homozygotes. In contrast, mini-white at 61E cannot be trans-inactivated by T(2;3)het; 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] and 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 heterochromatic insertion (Henkoff 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 1996), and recent genetic results have extended this interpretation to trans-inactivation of the nucleus (HENIKOFF et al. 1995) might be the basis for trans-inactivation in general.

We thank GEORGETTE SASS for insightful discussions and the Howard Hughes Medical Institute for support.

LITERATURE CITED


Communicating editor: J. A. BIRCHLER