A pairing-sensitive element that mediates trans-inactivation is associated with the Drosophila brown gene

Thomas D. Dreesen,1,2 Steven Henikoff,3,4 and Kate Loughney5

Basic Sciences Division and 3Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 USA

Position-effect variegation in Drosophila is the mosaic expression of a gene juxtaposed to heterochromatin by chromosome rearrangement. The brown (bw+) gene is unusual in that variegating mutations are dominant, causing "trans-inactivation" of the homologous allele. We show that copies of bw+ transposed to ectopic sites are not trans-inactivated by rearrangements affecting the endogenous gene. However, when position-effect variegation is induced on an ectopic copy by chromosome rearrangement, the allele on its paired homolog is trans-inactivated, whereas other copies of bw+ are not. This confirms that trans-inactivation is "chromosome local" and maps the responsive element to the immediate vicinity of brown. Subsequent P-transposase-induced deletions within the ectopic copy in cis to the rearrangement breakpoint caused partial suppression of trans-inactivation. Surprisingly, the amount of suppression was correlated with deletion size, with some degree of trans-inactivation persisting even when the P[bw+] transposon was completely excised. The chromosome-local nature of the phenomenon and its extreme sensitivity to small disruptions of somatic pairing leads to a model in which a regulator of the brown gene is inactivated by direct contact with heterochromatic proteins.

[Key Words: Position-effect variegation; Drosophila; somatic pairing; in vivo deletion mapping]

Received November 21, 1990; accepted December 18, 1990.

A common class of mutations induced by ionizing radiation in Drosophila are the variegating position effects. These are generally caused by chromosomal rearrangements placing euchromatic genes in the vicinity of heterochromatin, the compacted regions of chromosomes that remain condensed during most of the cell cycle (for review, see Spofford 1976; Henikoff 1990). The variegated phenotype is caused by reduction in gene expression in some cells but not in others, evidently as a result of transcriptional inactivation (Henikoff 1981; Rushlow et al. 1984; Henikoff and Dreesen 1989). Consistent with this interpretation, position-effect variegation of nearly all genes is recessive, implying that inactivation of one copy of a gene in cis has no effect on the copy in trans. However, variegated position effects on a few genes, including the brown (bw+) gene, are dominant (Muller 1932; Slatis 1955; Stern and Kodani 1955; Henikoff 1979, O'Donnell et al. 1989).

Recently, we showed that dominant position-effect variegation of brown involves a sharp reduction in mRNA accumulation from both copies of the gene, the copy in cis to the rearrangement breakpoint [cis-inactivation] and the copy in trans [trans-inactivation]. We proposed a somatic pairing model in which the brown gene region, inactivated by virtue of its juxtaposition to heterochromatin, could transmit the inactivated state to the copy of brown in trans. Evidence for this model was the sensitivity of trans-inactivation to chromosome configurations that are expected to disrupt somatic pairing in the vicinity of the brown gene.

The somatic pairing model makes several predictions. Copies of bw+ inserted at ectopic sites should not be trans-inactivated by variegating alleles at the normal locus, because pairing will not occur. However, a copy of bw+ inserted into the immediate vicinity of the brown locus itself might be trans-inactivated. In addition, induction of position-effect variegation on an ectopic copy of bw+ by chromosome rearrangement should cause trans-inactivation of its paired homolog if the hypothesized element that mediates the phenomenon is within the ectopically inserted segment. Here we show that these predictions are fulfilled and that the responsive element maps very close to or within the brown gene itself. Surprisingly, complete excisions and small deletions of the copy of bw+ in cis to the rearrangement breakpoint partially suppress trans-inactivation in a size-dependent manner. These results can be explained if
these lesions act to disrupt local somatic pairing necessary for trans-inactivation to occur. We propose that trans-inactivation results from contact between heterochromatin proteins present on the cis-inactivated chromosome and a positive regulator acting on the trans copy of the brown gene. This would represent a novel mechanism for gene inactivation.

Results

The functional brown gene lies within an 8.4-kb genomic segment

The brown gene had been cloned by chromosome walking from a microcloned library of the 59E chromosomal region. Its identity was established by localizing chromosome lesions in bw mutants to a region of DNA that gives rise to a pair of overlapping transcripts [Dreesen et al. 1988]. The predicted protein encoded by these transcripts showed striking similarity to that encoded by the Drosophila white and scarlet genes and to members of a family of active transport proteins. This finding was in accordance with the expected functions of brown, white, and scarlet in transport of pigment precursors [Sullivan and Sullivan 1975] and their hypothesized protein–protein interactions [Farmer and Fairbanks 1986]. A genomic clone [designated 1111 in Dreesen et al. 1988] derived from a cosmid library made from DNA of the wild-type Sevelen strain was used for further studies. The 8.4-kb EcoRI fragment that includes the entire coding region was subcloned into vectors for making nested deletions for sequencing and for P-element-mediated germ-line transformation.

Partial sequencing of the 8.4-kb insert indicated that the sequence corresponding to brown cDNAs is interrupted by 6 small introns, for an overall length of the transcription unit of ~3.2 kb [data not shown]. Figure 1 summarizes the relationship between this transcription unit and the 8.4-kb insert: 4.3 kb of flanking sequence lies upstream and 0.9 kb lies downstream of the brown transcription unit. Plasmids containing the 8.4-kb insert cloned into the pDm24 transformation vector were injected into flies for germ-line transformation. The resulting 12 transformants complemented bw null alleles, demonstrating that the functional brown gene lies within the 8.4-kb insert.

DNA from cosmid 1111 [Dreesen et al. 1988], with ~10 kb upstream and 20 kb downstream of the bw+ transcription unit [Fig. 1], was also injected, yielding a single transormant with a tandem double insertion at site 65F. Jumps to new sites induced by P- transposase were obtained by reversion of position-effect alleles described below. In addition, transposons carrying the 8.4-kb insert were similarly mobilized. In all, 36 independent single-insert transformants were chosen for further characterization.

The cytological localization of each insert is listed in Table 1. All flies carried the scarlet mutation, which eliminates the brown ommochrome pigments, leaving only the red and yellow pteridines. In this way, the genotype difference between bw+ and bw− could be scored as the phenotypic difference between scarlet and white, rather than dark red of wild-type flies and brown. The eyes of most transformants were orange when a single ectopic copy was present in females, corresponding to ~50% of the wild-type level of pigment (Table 1; Fig. 2a). In contrast, nontransformed bw+/bw−,st flies and flies carrying two transposed copies had scarlet eyes. The lower level of pigment attributable to a single copy of bw+ does not appear to result from exclusion of a cis-acting element; lines carrying single cosmid inserts showed similar reductions in pigment levels. Therefore, it is possible that the sub-wild-type levels of pigment in more than two-thirds of our lines is an inherent property of the parent chromosome from which our clones were derived. Regardless of the underlying cause of this uniform reduction in pigment, the observable difference between one- and two-dose flies allowed for lines to be easily made homozygous by inter se crosses (see Materials and methods).

Reduced levels of pigment attributable to one dose of bw+ allowed for detection of dosage compensation in most X-linked lines: Eyes of females were orange and males were red. In one-quarter of all lines, red eyes were seen for flies of both sexes with single doses of bw+; and in three autosomal lines, males showed more pigment than females. Such euchromatic position effects causing uniform changes in gene activity have been seen for other Drosophila genes introduced into many different sites [Spradling and Rubin 1983; Laurie-Ahlberg and Stam 1987].

Insertions of the brown gene at ectopic sites are not affected by rearrangements that strongly trans-inactivate an endogenous copy

To determine whether any of the ectopic copies of bw+ could be trans-inactivated, each of the 36 lines was examined in the presence of classical dominant bw+ alleles...
**Trans-inactivation element at the brown gene**

Table 1. *P[ bw +* ] single insert lines

| Map location* | Plasmid b | Source | Phenotype e | Female | Male | V21<sup>extreme</sup> variegation f |
|---------------|-----------|--------|-------------|--------|------|-----------------|
| 1C (X)        | L         | jump from 8C | r       | r      | no               |
| 3E (X)        | L         | jump from 8C | o       | r*     | no               |
| 6C (X)        | L         | jump from 8C | o       | r-o*e  | no               |
| 6E (X)        | L         | injection    | o       | r      | no               |
| 7D (X)        | L         | injection    | o       | r      | no               |
| 8C (X)        | L         | injection    | o       | r      | ND               |
| 12B (X)       | L         | jump from 8C | r       | r      | no               |
| 12D (X)       | L         | jump from 8C | o       | l      | no               |
| 18D (X)       | L         | injection    | o       | r      | no               |
| 18D (X) 1111  | L         | jump from V32| o       | r*     | no               |
| 19A (X)       | L         | jump from 8C | o       | r*     | no               |
| 19F (X)       | L         | jump from 8C | r-o     | r*     | no               |
| 27F (2L)      | R         | injection    | o       | r      | no               |
| 30B (2L)      | R         | injection    | o       | r      | ND               |
| 45B (2R)      | L         | jump from 8C | r       | r      | no               |
| 47A (2R)      | L         | injection    | r       | r      | no               |
| 49B (2R) 1111 | L         | jump from V8 | o       | o      | no               |
| 49F (2R)      | L         | jump from 8C | o       | o      | no               |
| 55C (2R)      | L         | jump from 8C | o       | o      | no               |
| 55E (2R)      | L         | jump from V32| o       | o      | no               |
| 59E (2R)      | R         | injection    | v       | v      | ND               |
| 60B (2R)      | L         | jump from 8C | o       | o      | no               |
| 62C (3L)      | L         | jump from 8C | o       | o      | ND               |
| 65D (3L)      | L         | injection    | o       | o      | no               |
| 65Fx2 (3L)    | L         | injection    | r       | r      | no               |
| 65Fx2 1111    | L         | loss from 65Fx2| o     | o      | no               |
| 66D (3L)      | L         | injection    | o       | o      | no               |
| 68B (3L)      | L         | jump from 8C | r-o     | r      | no               |
| 70D (3L)      | L         | jump from 8C | o       | o      | no               |
| 72B (3L)      | L         | jump from V21| o       | o      | no               |
| 79B (3L)      | L         | jump from 8C | o       | o      | no               |
| 79D (3L)      | L         | injection    | r       | r      | no               |
| 82C (3R)      | L         | jump from 8C | r       | r      | no               |
| 88F (3R)      | L         | jump from 8C | o       | o      | ND               |
| 92C (3R)      | L         | injection    | o       | o      | yes              |
| 94C (3R)      | L         | injection    | o       | r      | no               |
| 94D (3R)      | L         | jump from V21| r       | r      | no               |

*Salivary chromosome site (arm).

bFor maps of L, R, and 1111, see Fig. 1.

Based on phenotypes of single copies of *P[ bw + ]* in backgrounds of *bw<sup>P</sup>/bw<sup>P</sup>;st, bw<sup>P</sup>/bw;<sup>P</sup>;st, bw<sup>P</sup>/P<sub>m</sub>;st, and P<sub>m</sub>/bw;<sup>P</sup> at 18°C, with some inserts not tested for all combinations. Phenotypes are red (r), orange (o), red-orange (r-o), lethal (l) and variegated (v).

dPresence of spots or clones in *bw°; st* V21<sup>e</sup>/st mutant background, at 18°C. V21<sup>e</sup> (V21 e), is a P-transposase-induced derivative of V21 selected for extreme enhancement of cis-inactivation, which also shows enhanced trans-inactivation (Table 2). No alteration was detected within the *P[ bw + ]* transposon of V21<sup>e</sup>. (ND) Not determined.

dosage-compensated in males.

This insertion [R26] is inseparable from *bw°*. Therefore, phenotypic tests could be carried out only in *bw + P[ bw + ]*/bw;<sup>D</sup>;st and *bw + P[ bw + ]*/P<sub>m</sub>;st flies.

65Fx2 = tandem insertion of plasmid 1111; 65Fx1 = P-transposase-induced single-copy derivative.

In 35 of these lines, no mutant spots or clones characteristic of trans-inactivation were seen (Table 1). Even a copy of *bw°* at 60B, just distal to brown by a distance corresponding to 1–2% of the length of chromosome 2, was not detectably trans-inactivated. Similarly, trans-inactivation was not detected for cosmid insertions, not even for a copy on the male X chromosome, which lacks a homolog able to compete for pairing with sequences flanking the transposon. These results are consistent with the possibility that the *bw°* copy affected in trans must be very close to the 59E brown chromosomal region, that is, that trans-inactivation is a chromosome–local phenomenon.

One transformant, called R26, was exceptional. The *bw°* insert was strikingly trans-inactivated when heterozygous with *bw°* rearrangements (Fig. 2b). In R26, the
inserted transposon colocalizes with the resident \(bw^+\) copy at 59E1-2 by in situ hybridization (Table 1) and appears to be inseparable from it by recombination (<0.03 cM). This suggests further that trans-inactivation is chromosome local. However, we have not proven that the transposon in R26 encodes an active \(bw^+\) gene product: Attempts to separate the transposon from the resident \(bw^+\) copy by recombination or mobilization or to clone the region intact from phage and cosmid libraries constructed from R26 flies have not succeeded (data not shown).

The trans-inactivation phenomenon can be reproduced at sites of \(P[ bw^+ ]\) transposons

If sequences necessary for trans-inactivation are present on the \(P[ bw^+ ]\) segments used for transformation, then it should be possible to reproduce the trans-inactivation phenomenon at sites of these transposons (Fig. 3a). Two insertions into chromosome 3 were chosen: \(P[ bw^+ (92C) ]\), which includes the 8.4-kb EcoRI \(bw^+\) fragment inserted into 92C, and the tandem cosmid insertion of 1111 at 65F. Males homozygous for either of these insertions and for a null \(bw^-\) allele \((bw^0)\) were X-irradiated, crossed to \(bw^0\) females, and their progeny screened for variegating red pigment (cis-inactivation). Four mutants were obtained. One, called V21, showed a classical "clonal" phenotype (Fig. 2c) associated with an inversion placing heterochromatin just proximal to the \(P[ bw^+ (92C) ]\) transposon (Table 2). The other three were translocations placing either X- or Y-derived heterochromatin adjacent to the tandem \(P[ bw^+ (65F) ]\) transposons. The phenotypes of these ranged from very strong "pepper and salt" cis-inactivation of both tandem \(bw^+\) copies for V32 (Fig. 2d) to an intermediate degree of cis-inactivation in V14 (Fig. 2e), to a barely detectable level of cis-inactivation in V8 (Fig. 2i). Such a wide range of phenotypes is typically seen in screens for cis-inactivation of the brown gene (e.g., Slatis 1955). That these are examples of position–effect variegation, and not permanent alterations in the gene, was demonstrated by selecting for transposase-mediated revertants to nonvariegating \(bw^+\),
which for V21, V32, and V8 were jumps to new sites (Table 1; data not shown).

All four variegating position effects were tested for their ability to trans-inactivate copies of bw+. V21, the inversion-associated derivative of P[bw+(92C)], trans-inactivated P[bw+(92C)] (Fig. 2g) but had no detectable effect on any other bw+ copy. A more strongly variegating derivative of V21, V2Iextreme, trans-inactivated P[bw+(92C)] more strongly but had no effect on any other P[bw+] insertion (Table 1). These results demonstrate that the phenomenon of dominant position-effect variegation can be reproduced at the site of a P[bw+] transposon and is chromosome local at that site. Both V14 and V32, translocations derived from P[bw+(65F)], trans-inactivated P[bw+(65F)] but not P[bw+(92C)]. This suggests that the ability to trans-inactivate in a chromosome-local manner is a property of the brown gene segment and not the particular position into which it is inserted. Special conditions were necessary to detect trans-inactivation caused by V8, an example of weak cis-inactivation of the tandem duplication: Trans-inactivation of a single P[bw+] copy at 65F could be observed in certain P-transposase-induced V8 derivatives lacking both P[bw+] copies in cis (V8–24 in Table 2). Thus, four of four variegating derivatives of P[bw+] transposons selected for cis-inactivation are able to trans-inactivate the gene on the parent transposon.

Deletions induced in V21 cause variable suppression of trans-inactivation

Since the V21 inversion causes classical dominant position-effect variegation at the site of a transposon with only 8.4 kb of DNA derived from the brown region, we used this chromosome to carry out further studies of trans-inactivation. We reasoned that deletion analysis of the transposon carrying the bw+ gene in V21 should allow us to identify DNA sequences present on the chromosome that might be required for trans-inactivation of P[bw+]. (Fig. 3b).

Deletions generated in the germ lines of V21 flies using P-element transposase encoded on the P[Δ2,3(99B)] third chromosome were recovered as bw+-derivatives of V21. Fifty-one individual V21[P[bw+]] lines were established and examined by genomic Southern blotting to determine what portion of the transposon had been deleted. All were detectably deleted or rearranged within the bw+-coding region (Fig. 4). Twenty deletions fell completely within the 8.4-kb EcoRI fragment, and 15 of these were chosen for further analysis. For each, the remaining portion of the 8.4-kb fragment was rescued as a plasmid, and the deletion endpoints were mapped. In addition, the ability of V21[P[Δbw]] lines to trans-inactivate the P[bw+(92C)] transposon was scored. A visual scoring scheme was employed, with 6 corresponding to a

Figure 3. Experimental strategies for identifying and mapping the cis-acting genetic element responsible for trans-inactivation. (a) Phenotypically wild-type flies carrying the brown gene and flanking sequences on chromosome 3 transposons (P[bw+]) were x-irradiated and screened for examples of position-effect variegation (cis-inactivation), then tested for dominance over the parent transposon (trans-inactivation). Wild-type and variegated phenotypes are indicated at right. (b) A trans-inactivating example (V21) was mutagenized using P-element-encoded transposase, leading to deletions and excisions. These were also tested for trans-inactivation. Pairs of open boxes represent homologous chromosomes, with centromeres as circles and pericentric heterochromatin as solid boxes.

Figure 4. Schematic diagram depicting the classes of P[bw+] lesions obtained by mutagenizing V21 with P-element-encoded transposase. The parent transposon in V21 is shown at top. The different classes of deletions (Δ) are shown along with the number of independent lines falling into each class indicated on right. Rearrangements are lesions involving the brown gene that appeared to be more complex than simple deletions or fusions with flanking sequences.
Table 2. Position-effect variegation on P[bw+] transposons

| Chromosome | P[bw+] and lesion | Cis-inactivation phenotype | Trans-inactivation | (Score)|
|------------|-------------------|---------------------------|-------------------|-------|
| V21        | 92C In(3)80-81,92C | ~50% mutant spots and clones | yes               | (2)   |
| V21*extreme| P[bw+] A of V21   | ~99% mutant spots and clones | yes               | (0)   |
| V32        | 65Fx2 T(X;3)20F,65F | ~99% mutant spots | yes               | (4)   |
| V14        | 65Fx2 T(X;3)20F,65F | ~20% mutant spots | yes               | (3)   |
| V8         | 65Fx2 T(Y;3)Y,65F | ~20 mutant spots/eye | no                | (6.0) |
| V8–24      | P[Δbw] from V8    | null                      | yes               | (5–6) |

*aScored for heterozygotes with a wild-type chromosome 3 in a bwD;st background.
*bScored for V21 and V21*extreme using heterozygotes with P[bw+] [92C] and for other chromosomes using P[bw+] [65Fx1]. The scores are based on the scale described in Materials and methods and reflect contributions from both the cis and trans copies of the gene.
*cRecessive lethal or semilethal.
*dPosition effect confirmed by mobilization of P[bw+] to new sites with concomitant restoration of uniform pigmentation.
*eMale-sterile.

The phenomenon indistinguishable from P[bw+] [92C]/− and 0 to a phenotype in which ~50% or more of the pigment cells in the eye appeared to be inactivated for expression of P[bw+]. To compare overall scores among lines, care was taken to maintain uniform genetic and environmental conditions.

Figure 5a shows the endpoints and average score for each deletion derived from V21. Trans-inactivation was detected in every case, even when all sequence between the P-element ends was excised. In addition, the degree of trans-inactivation varied from line to line over a wide range. When scores are plotted as a function of deletion length, a clear correlation is seen, with progressively larger deletions showing less and less trans-inactivation (Fig. 5b). The smallest degree of trans-inactivation is associated with complete excision of the transposon in V21 and possibly of flanking DNA sequences as well. The most extreme trans-inactivation is associated with the smallest V21[P(Δbw)] deletion, which has a lower score than no deletion at all. It should be noted that P[bw−] derivatives of V21 are expected to have lower scores than V21 itself, because of the contribution to the overall levels of pigment from the P[bw+]/copy present on V21. Nevertheless, V21[P(Δbw)] deletions that are only a few kilobases in size lead to higher pigment levels than V21, indicating suppression of trans-inactivation. The extreme sensitivity of the phenomenon to such small deletions, and the striking correlation between the size of a deletion and the degree to which trans-inactivation was suppressed, was quite unexpected.

Discussion

Proposed mechanism for trans-inactivation

For nearly 60 years, attempts have been made to explain the enigmatic dominance of bw+ rearrangements (Müller 1932; Ephrussi and Sutton 1944; Stern and Heidenthal 1944; Lewis 1950; Spotford 1976; Frankham 1988; Henikoff and Dreesen 1989). Recently, we presented evidence for the somatic pairing dependence of this phenomenon and hypothesized the existence of a pairing-sensitive genetic element in the approximate vicinity of the brown gene (Henikoff and Dreesen 1989). This element was termed a “transceiver” to reflect its presumed action: When present on the chromosome in trans to the heterochromatic breakpoint, the element could receive the inactivating influence of heterochromatin. However, the location of the transceiver relative to the brown gene could not be determined with precision. In work reported here, we have caused trans-inactivation to occur at the site of a transposon that includes the brown gene and only short flanking sequences. This indicates that the hypothesized transceiver is linked very tightly to the gene on a molecular scale.

What is the nature of the transceiver? A simple model is that the transceiver is itself responsible for an unusual degree of somatic pairing in the brown region. Such a site of unusual pairing could act as a conduit for the inactivating influence of heterochromatin to pass across homologs as suggested previously (Henikoff and Dreesen 1989). The uncertainty in breakpoint location is indicated by stippling. Numbers associated with each deletion and excision represent average pigment scores for 18–116 flies (mean = 70). The first two scores are for complete (but likely imprecise) excisions of the transposon, and the “no deletion” score is for V21[P[bw+]]/P[bw+ {92C}]. The uncertainty in breakpoint location is indicated by stippling. Numbers associated with each deletion and excision represent average pigment scores for 18–116 flies (mean = 70). The first two scores are for complete (but likely imprecise) excisions of the transposon, and the “no deletion” score is for V21[P[bw+]]/P[bw+ {92C}]. In each case, the average score for P[bw+] [92C]/− siblings was 6.0. (b) The same data are plotted as a function of deletion or excision size.
present a possible molecular model for functions as a positive regulator of have “spread” from the junction between heterochromatin {Fig. 6). We propose that the transceiver normally inactivation in the same way. To illustrate this, we present a possible molecular model for trans-inactivation [Henikoff and Dreesen 1989]. For example, Su(Pm), a tandem duplication of the brown region that disrupts somatic pairing in polytene chromosomes, partially suppresses trans-inactivation. The much smaller scale of lesions involving the brown gene in this study might be effective in suppressing trans-inactivation in the same way. To illustrate this, we present a possible molecular model for trans-inactivation [Fig. 6]. The figure shows that the transceiver normally functions as a positive regulator of brown expression. Its novel feature is its sensitivity to pairing with a homolog that is complexed with heterochromatin proteins that have “spread” from the junction between heterochromatin and euchromatin. When one copy of the brown gene is cis-inactivated by heterochromatin and is in a region that can pair with its homolog, the regulator of bw expression on the homolog might make frequent contact with heterochromatin proteins and thus be prevented from normal functioning [Fig. 6a]. Alternatively, this contact might prevent a trans-acting factor from binding to the regulator. In the case of small deletions, a segment loops out so that contact of the regulator with heterochromatin proteins on the deleted homolog occurs less frequently, leading to partial suppression of trans-inactivation [Fig. 6b]. In the case of complete excisions, the entire gene region loops out in nearly all cells, although very occasionally contact occurs with heterochromatin proteins bound to regions flanking the excised copy [Fig. 6c]. Thus trans-inactivation can occur at a detectable level following complete excision of the brown gene in cis [Fig. 5a]. Also, suppression of trans-inactivation can be more effective for large deletions than for small [Fig. 5b]. Since trans-inactivation would involve any number of heterochromatin proteins in the vicinity of the brown gene, this model explains how a single bw V rearrangement can trans-inactivate multiple copies of the brown gene in individual cells when flanking homologous sequences are sufficient to allow somatic pairing to occur [Henikoff and Dreesen 1989].

One might expect from this model that deletions extending into the upstream region of the brown gene would be more effective in suppressing trans-inactivation than similarly sized deletions broken entirely within the coding region. One possible explanation for our inability to detect such a difference is the limited precision of our assay for dominant variegation, since standard deviations averaged 0.6 and scores ranged from 2.0 to 4.8, we were unable to distinguish minor differences between points and were only able to distinguish the consistent trend based on all of the points. Furthermore, we were limited to deletions that eliminated bw + expression but did not extend into the adjacent vector. Therefore, most of the larger deletions extended well upstream, whereas most of the smaller deletions were entirely within the gene. Another possible explanation for failure to better localize the pairing-sensitive region is that disruption of pairing might cause local asynapsis beyond the deletion breakpoints, as is seen in polytene chromosome squashes. Suppression of trans-inactivation would be determined primarily by “slack” in the loop, which has more freedom to escape contact with heterochromatic proteins for larger loops, as illustrated in Figure 6.

Dominant variegation is unusual among Drosophila genes susceptible to position-effect variegation. Even in the case of the brown gene, the phenomenon might be limited to eyes pigment cells, since brown variegation in other tissues expressing the gene appears to be recessive [Lindsley and Grell 1968; K. Loughney and S. Henikoff, unpubl.]. Perhaps the difference between susceptibility and resistance to trans-inactivation lies in some special property of the transceiver. For example, the pairing-sensitive component of trans-inactivation might be an eye-

**Figure 6.** A model for trans-inactivation of the brown gene. (a) The transceiver (open circle) is a positive regulator of bw + that can be prevented from normal functioning (stippled circle) whenever intimate contact is made with heterochromatin proteins (stippled octagons) that spread from the V21 breakpoint. (b and c) Deletions and excisions within P[bw +] on the cis-inactivated homolog cause the trans copy to loop out. As a result, the bw + gene is more frequently active, leading to fewer mutant spots in the eye. The larger the loop, the less likely it is that intimate contact will occur between heterochromatin proteins and the transceiver. Triangles represent 5’ and 3’ P-element ends flanking the copy of bw + inserted at 92C.
specific transcription factor that is somehow prevented from assuming an active conformation. In contrast, the white gene, which never shows dominant variegation, would not be regulated by this particular transcription factor, even though it is expressed in the same cells as the brown gene and encodes a related protein.

The above mechanism supposes that cis-inactivation seen in position-effect variegation is caused by heterochromatin compaction of chromatin. However, another model for position-effect variegation proposes that cis-inactivation is a consequence of the altered nuclear position of a gene involved in a variegating rearrangement (Ephrussi and Sutton 1944; Wakimoto and Hearn 1990). Synaptic forces might cause the repetitive sequences in heterochromatin to coalesce into a heterochromatin-rich nuclear compartment, seen as the chromocenter in polytene cells. Thus, euchromatic genes placed next to heterochromatin repeats would be dragged into or adjacent to this compartment, possibly excluding their regulatory sites from euchromatic components necessary for expression. This model is not inconsistent with our results. The same synaptic forces that drag the rearranged copy of the brown gene into the heterochromatin-rich compartment might also drag its paired homolog there, making both unavailable for a necessary trans-acting factor. It is possible that the looping out caused by deletions of bw+ in V21 could affect the gene’s location within the nucleus, allowing it to become more frequently accessible to the factor.

It is more difficult to account for our results in terms of two other models proposed to explain position-effect variegation. Frankhain hypothesized that dominant variegation is caused by transcripts initiating within heterochromatin, leading to inactivation by antisense RNA hybridization (Frankham 1988). Our previous failure to detect antisense transcripts in strongly variegating rearrangements is inconsistent with this model (Henikoff and Dreessen 1989). The antisense RNA model also cannot easily account for the chromosome–local nature of the phenomenon demonstrated here, nor for the persistence of dominant variegation following complete excision of the entire wild-type brown gene from which antisense RNA is presumably made. A somatic gene loss model for position-effect variegation, originally proposed by Schultz and recently reformulated by Karpen and Spradling, also does not easily account for dominant variegation (Schultz 1936; Karpen and Spradling 1990). If gene loss were responsible for the variegation in V21, it is difficult to see how V21[PΔbw] deletions could lead to suppressed phenotypes in a size-dependent manner. That is, by the somatic gene loss model, chromosomes carrying both 0.5- and 5-kb germ-line deletions within V21[Pbw] would subsequently undergo somatic loss of the entire brown gene region; thus, identical variegating phenotypes would be expected. Yet we observed a dramatic effect of deletion size on phenotype (Fig. 5b).

Relationship of trans-inactivation to other phenomena
The somatic pairing dependence of trans-inactivation places it in a large class of pairing- or proximity-dependent phenomena in Drosophila (for review, see Judd 1988; Wu and Goldberg 1989; Ashburner 1990). Many examples of these phenomena are affected by mutations at the zeste locus. The zeste protein has been shown to act as a transcription factor and to self-associate in vitro, consistent with models for activation of transcription across paired homologs (Zachar et al. 1985; Benson and Pirrotta 1988; Wu and Goldberg 1989). Although trans-inactivation of brown is not affected by zeste alleles (S. Henikoff, unpubl.), there are intriguing parallels between this phenomenon and zeste+-dependent repression of paired copies of the white gene, a phenomenon that also is suppressed by local disruption of synopsis (Gubb et al. 1990). The mechanism of repression is thought to be interference with white gene transcription by large aggregates of mutant zeste+ protein binding just upstream of the gene (Bickel and Pirrotta 1990). Perhaps trans-inactivation of brown occurs by an analogous mechanism. The hypothesized brown transcription factor might specifically associate with a particular heterochromatin protein across paired homologs, drawing the trans copy of the gene into an inactivating heterochromatic complex (Fig. 6). What would then be unusual about the brown gene is the tendency of this transcription factor to associate with a heterochromatin protein. Such a heterologous association between a transcription factor and a protein that can interfere with its function is thought to occur in other situations (Benezra et al. 1990; Ellis et al. 1990; Garrell and Modolell 1990).

We would not be surprised if features of trans-inactivation are involved in phenomena seen in other organisms. In fact, Laird has proposed that Huntington’s disease is an example of dominant position-effect variegation, suggesting that the brown gene might provide a model system for understanding the genetic basis of this disease (Laird 1990).

Materials and methods
Drosophila melanogaster stocks and plasmids
Mutant alleles and chromosomes used in this study [bw, bw*, st, Sb, Pm (=[In(2LR)bw+]1] and In[3R]Payne] are described by Lindsley and Grell (1968). bw, bw*, and st are null alleles, such that bw; st and bw; st flies have white eyes (Slatis 1955). P{py Δ2,3(99B)} is described by Robertson et al. (1988). Cosmid clone 1111 (Dreessen et al. 1988) consists of a segment derived from 59E2 of the wild-type Sevelen strain inserted into Drosophila transformation vector cosmPneo (Steller and Pirrotta 1985). The Drosophila transformation vector pDm24 was described by Misser and Rubin (1987). Both vectors include a fusion construct between the D. melanogaster hsp70 promoter and the Escherichia coli neomycin-resistance gene.

Germ-line transformations
Plasmids were injected into preblastoderm embryos of the genotype ry606 P{py Δ2,3(99B)} using standard procedures (Spradling 1986). G0 adults were crossed to bw/+; st mates, and their G1 progeny screened for visible red eye pigment. Because the bwD allele is null in cis and is essentially fully dominant in a chro-
mosome-local manner, bwD/+ eyes are brown, whereas bwD/+ P[bw+] transfectants have red pigment, with frequent sectors resulting from somatic activity of P[ry·Δ2.3(99B)]-encoded transposase. C5 transfectants were crossed to bwD,st males, and their progeny crossed inter se. In most cases, selection of the nonsectored bright red-eyed (st+) progeny allowed homozygous lines to be set up, since these indicated the presence of two doses of P[bw+]. Some insertions were associated with lethality: These were maintained by selection of orange- or red-eyed progeny. Transposons were mapped by in situ hybridization (Simon et al. 1985), and multiple insertion lines were discarded.

P-transposase-induced mobilizations and deletions

All mobilizations and deletions were carried out in a bwD, st or bwD,st (white-eyed) background, such that the only eye pigment was contributed by the P[bw+] transposon. An X-linked lethal insertion line, P[bw+8E was made homozygous for P[ry·Δ2.3(99B)], such that progeny consisted of white-eyed females and males, and females with sectored eyes. For each mobilization, a single, sectored female was placed in a vial, and her progeny and grandprogeny (if necessary) were examined for the appearance of pigmented males. Each of these represented a mobilization event to the wild-type X or to an autosome. Other mobilization events were obtained from variegated derivatives: T(Y;3)V8 was crossed to females x pendential deletions of P[bw+] were obtained by crossing sites of insertion were mapped by in situ hybridization. Independent deletions of P[bw+] were obtained by crossing bwD,st females × bwD,st In(3) V21 Sb/st P[ry·Δ2.3(99B)] single males and selecting single white-eyed Sb flies from each vial (~4% of the progeny). Balanced stocks were maintained as bwD,st Sb V21[P[bw+]/st In]3RPayne.

X-ray mutagenesis

Homzygous bwD, st P[bw+](92C)] and bwD, st P[bw+](65Fx2)] males were aged ~3 days, irradiated with 4000 rad X-rays, and mated to bwD, st virgin females. A total of 8,552 bwD, st P[bw+](92C)]/st progeny were examined for nonuniform pigmentation. The four heritable examples of variegated expression were repeatedly outcrossed to bwD, st. Genetic mapping was used to determine linkage and in situ hybridization to confirm that each is associated with a heterochromatic breakpoint adjacent to the P[bw+] transposon, as is expected for position-effect alleles of bw and other genes [Slatis 1955; Spofford 1976].

Mapping and scoring of deletions

Individual V21[P[bw+] lines were used to prepare DNA for genomic Southern blotting (Bender et al. 1983, modified by J. Hirsh) to determine what portions of the transposon had been deleted. Those with breakpoints internal to the 8.4-kb insert were used for plasmid rescue (Wilson et al. 1989). All other cloning and DNA sequencing procedures were carried out according to standard protocols [Sambrook et al. 1989].

Scoring of V21[PΔbw]/P[bw+](92C)] flies for levels of trans-inactivation was carried out under carefully controlled conditions to minimize experiment-to-experiment and fly-to-fly variability. Since trans-inactivation by V21 and V21+ is strongly suppressed at temperatures above ~20°C, flies were cultured at 18°C. A uniform genetic background was maintained by using the same laboratory stocks for crosses. [No evidence of segregating modifiers that might have influenced pigment scores was detected in any of the experiments reported here.] Because very small pigment spots are counted more easily in an st+ than in an st- background, st+ was introduced in the cross bwD,st+ P[bw+](92C)] females × bwD,st+ Sb V21[P[bw+]/In]3RPayne males. The resulting Stubble progeny were collected over a 6- to 8-day period and aged for about a week before scoring. To avoid inadvertent bias, labels were covered after collection and only uncovered after scoring. The following scoring scale was used: 6 = 0–1 mutant spots per eye; 5 = 1–10 spots, 4 = 10–30 spots, 3 = ~30 spots to ~25% of the eye mutant; 2 = ~10–25% mutant; 1 = ~25–50% mutant; and 0 = ~50% mutant. Each eye was given an integral score. Only scores from females are reported, since V21 trans-inactivation in males is less extreme, typically averaging one point higher in score.

Acknowledgments

We thank Cosette D. S. LeCiel for technical assistance in carrying out the x-ray screen and in performing the in situ hybridizations. We thank Mohammad K. Eghtedarzadeh for performing injections for germ-line transformation. We also thank our many colleagues who provided helpful comments on the work as it progressed and on the manuscript. This work was supported by a grant from the National Science Foundation.

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T D Dreesen, S Henikoff and K Loughney

*Genes Dev.* 1991, 5:
Access the most recent version at doi:10.1101/gad.5.3.331

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