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

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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.

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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

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these lesions act to disrupt local somatic pairing necessary for *trans*-inactivation to occur. We propose that *trans*-inactivation results from contact between heterochromatic 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*⁻ mutations 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 *Eco*RI 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 transformant 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 genotypic 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*^V al-

Figure 1. Maps of the *bw*⁺ transcription unit and plasmids used for transformation. The exon-intron structure of the gene is shown, with solid segments representing the protein-coding region and arrows pointing to the polyadenylation sites. (L and R) Plasmids with the same 8.4-kb *Eco*RI fragment in two different orientations. The orientation of 1111 within cosPneo is unknown. Solid segments of the plasmid maps represent vector, with embedded triangles indicating the approximate locations of the 5' and 3' P-element ends. Arrows on the plasmid maps show the direction of *brown* gene transcription. [E] *Eco*RI site.

Table 1. *P[bw⁺]* single insert lines

Map location ^a	Plasmid ^b	Source	Phenotype ^c		V21 ^{extreme} variegation ? ^d
			female	male	
1C (X)	L	jump from 8C	r	r	no
3E (X)	L	jump from 8C	o	r ^e	no
6C (X)	L	jump from 8C	o	r-o ^e	no
6E (X)	L	jump from 8C	o	l	no
7D (X)	L	injection	o	r ^e	no
8C (X)	L	injection	o	l	ND
12B (X)	L	jump from 8C	r	r	no
12D (X)	L	jump from 8C	o	l	no
18D (X)	R	injection	o	r ^e	no
18D (X)	1111	jump from V32	o	r ^e	no
19A (X)	L	jump from 8C	o	r ^e	no
19F (X)	L	jump from 8C	r-o	r ^e	no
27F (2L)	R	injection	o	r	no
30B (2L)	R	injection	o	o	ND
45B (2R)	L	jump from 8C	r	r	no
47A (2R)	L	injection	r	r	no
49B (2R)	1111	jump from V8	o	o	no
49F (2R)	L	jump from 8C	o	o	no
55C (2R)	L	jump from 8C	r	r	no
55F (2R)	1111	jump from V32	o	o	no
59E ^f (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 ^g (3L)	1111	injection	r	r	no
65Fx1 ^g (3L)	1111	loss from 65Fx2	o	o	no
66D (3L)	L	injection	o	o	no
68B (3L)	L	jump from 8C	o	o	no
70D (3L)	L	jump from 8C	r-o	r	no
72B (3L)	L	jump from V21	o	o	no
79B (3L)	L	jump from 8C	o	o	no
79D (3L)	R	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)	R	injection	o	r	no
94D (3R)	L	jump from V21	r	r	no

^aSalivary chromosome site (arm).^bFor maps of L, R, and 1111, see Fig. 1.^cBased on phenotypes of single copies of *P[bw⁺]* in backgrounds of *bw^D/bw^D;st*, *bw^D/bw;st*, *bw^D/Pm;st*, and *Pm/bw;st* 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^D;st Sb V21^e/st* mutant background, at 18°C. V21^{extreme} (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^e. (ND) Not determined.^eDosage-compensated in males.^fThis insertion (R26) is inseparable from *bw⁺*. Therefore, phenotypic tests could be carried out only in *bw⁺ P[bw⁺]/bw^D;st* and *bw⁺ P[bw⁺]/Pm;st* flies.^g65Fx2 = tandem insertion of plasmid 1111; 65Fx1 = P-transposase-induced single-copy derivative.

leles. 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^V* rearrangements (Fig. 2b). In R26, the

Figure 2. Eyes of transformed flies in a *st* mutant background. Flies are homozygous for *bw^D* at the *brown* chromosomal locus except as noted. *P[bw⁺]* is *P[bw⁺](92C)*, the third chromosome parent of V21. Minus (–) designates a third chromosome that lacks a transposon. (a) *P[bw⁺]* transformants; (b) the exceptional R26 transformant; (c–f) *cis*-inactivation of *P[bw⁺]* copies in position–effect variegation lines; (g) *cis*- and *trans*-inactivation involving V21.

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⁺* frag-

ment 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^D*) were X-irradiated, crossed to *bw^D* 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. 2f). Such a wide range of phenotypes is typically seen in screens for *cis*-inactivation of the *brown* gene (e.g., Slatkin 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⁺*,

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.

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, V21^{extreme}, *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 se-

lected 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 *Eco*RI 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(Δ*bw*)] lines to *trans*-inactivate the P[*bw*⁺(92C)] transposon was scored. A visual scoring scheme was employed, with 6 corresponding to a

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	<i>Cis</i> -inactivation phenotype ^a	<i>Trans</i> -inactivation?	(Score) ^b
V21	92C In(3)80–81;92C ^c	~50% mutant spots and clones ^d	yes	(2)
V21 ^{extreme}	$P[bw^+]$ Δ of V21 ^c	~99% mutant spots and clones ^d	yes	(0)
V32	65Fx2 T(X;3)20F;65F ^c	~99% mutant spots ^d	yes	(4)
V14	65Fx2 T(X;3)20F;65F ^{c,e}	~20% mutant spots	yes	(3)
V8	65Fx2 T(Y;3)Y;65F	~20 mutant spots/eye ^d	no	(6.0)
V8–24	$P[\Delta bw]$ from V8	null	yes	(5–6)

^aScored for heterozygotes with a wild-type chromosome 3 in a $bw^D;st$ 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.

phenotype 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 $P[bw^+]$ (see Materials and methods). 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[$P[\Delta bw]$] 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[$P[\Delta bw]$] 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^V rearrangements (Muller 1932; Ephrussi and Sutton 1944; Stern and Heidenthal 1944; Lewis 1950; Spofford 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

Figure 5. (a) Breakpoint map of V21[$P[\Delta bw]$] deletions within the 8.4-kb *EcoRI* bw^+ fragment and eye pigment phenotypes of heterozygotes with $P[bw^+ (92C)]$. 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.

1989). Indeed, our ability to reproduce the phenomenon at sites of *bw*⁺ transposons is consistent with this model. However, loss of the pairing site in *cis* to the rearrangement breakpoint would be expected to abolish *trans*-inactivation. The fact that complete excisions of the copy of the transposon in *cis* to the rearrangement breakpoint only partially suppressed *trans*-inactivation is inconsistent with this model. Furthermore, the correlation between suppression and the size of deletions within this transposon, rather than between suppression and the loss of a specific site or sites, rules out any simple pairing site hypothesis.

Alternatively, these unexpected results might be explained by supposing that suppression occurs by local disruption of pairing between the transceiver and the heterochromatinized homolog on a molecular scale. Local disruption of pairing on a cytological scale was previously proposed as an explanation for suppression of *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). We propose 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 heterochromatic proteins that have "spread" from the junction between heterochroma-

tin 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 heterochromatic 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 heterochromatic 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 heterochromatic 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 of a number of heterochromatic 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 eye 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 heterochromatic 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 heterochromatic 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 heterochromatic 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 heterochromatic repeats would be dragged into or adjacent to this compartment, perhaps 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. Frankham 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 Dreesen 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[P(Δ *bw*)] 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[P(*bw*)] 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*¹-dependent repression of paired copies of the *white* gene, a phenomenon that also is suppressed by local disruption of synapsis (Gubb et al. 1990). The mechanism of repression is thought to be interference with *white* gene transcription by large aggregates of mutant *zeste*¹ 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 heterochromatic 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 heterochromatic 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*^D, *st*, *Sb*, *Pm* [= In(2LR)*bw*^{V1}] and In(3R)Payne) are described by Lindsley and Grell (1968). *bw*, *bw*^D, and *st* are null alleles, such that *bw*;*st* and *bw*^D;*st* flies have white eyes (Slatis 1955). *ry*⁵⁰⁶ P[*ry*⁺ Δ 2,3(99B)] is described by Robertson et al. (1988). Cosmid clone 1111 (Dreesen et al. 1988) consists of a segment derived from 59E2 of the wild-type Sevelen strain inserted into *Drosophila* transformation vector cosPneo (Steller and Pirrotta 1985). The *Drosophila* transformation vector pDm24 was described by Mismar 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 *ry*⁵⁰⁶ P[*ry*⁺ Δ 2,3(99B)] using standard procedures (Spradling 1986). G₀ adults were crossed to *bw*^D;*st* mates, and their G₁ progeny screened for visible red eye pigment. Because the *bw*^D allele is null in *cis* and is essentially fully dominant in a chro-

mosome-local manner, $bw^D/+$ eyes are brown, whereas $bw^D/+$ P[bw^+] transformants have red pigment, with frequent sectors resulting from somatic activity of P[ry^+ - $\Delta 2,3(99B)$]-encoded transposase. G_2 transformants were crossed to $bw^D;st$ mates, 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 $bw^D;st$ or $bw^-;st$ (white-eyed) background, such that the only eye pigment was contributed by the P[bw^+] transposon. An X-linked lethal insertion line, P[bw^+] ΔE , was made homozygous for P[ry^+ - $\Delta 2,3(99B)$], such that progeny consisted of white-eyed females and males, and females with sector eyes. For each mobilization, a single, sector eye 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 P[ry^+ - $\Delta 2,3(99B)$]; male progeny were mated to $bw^D;st$, and their daughters were examined for pigment, indicating a jump from the T[Y;3]. In[3]V21 and T[X;3]V32 were crossed to P[ry^+ - $\Delta 2,3(99B)$], and their progeny were examined for nonvariegating and nonsectored phenotypes, indicating relief of *cis*-inactivation caused by a jump to a new site. Selected chromosomes were made homozygous, and new sites of insertion were mapped by in situ hybridization. Independent deletions of P[bw^+] were obtained by crossing $bw^D;st$ females $\times$ $bw^D;st$ In[3]V21 Sb/ st P[ry^+ - $\Delta 2,3(99B)$] single males and selecting single white-eyed Sb flies from each vial (~4% of the progeny). Balanced stocks were maintained as $bw^D;st$ Sb V21[P[bw^+]/ st In[3R]Payne.

X-ray mutagenesis

Homozygous $bw^D;st$ P[bw^+](92C)] and $bw^D;st$ P[bw^+](65F $\times$ 2)] males were aged ~3 days, irradiated with 4000 rad X-rays, and mated to $bw^D;st$ virgin females. A total of 38,622 $bw^D;st$ P[bw^+](92C)]/ st progeny and 14,752 $bw^D;st$ P[bw^+](65F $\times$ 2)]/ st progeny were examined for nonuniform pigmentation. The four heritable examples of variegated expression were repeatedly outcrossed to $bw^D;st$. 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[P[Δbw]]/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 vari-

ability. Since *trans*-inactivation by V21 and V21^e 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 $bw^D;st^+$ P[bw^+](92C)] females $\times$ $bw^D;st$ Sb V21[P[Δbw]]/In[3R]Payne 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 ~10% 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.

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References

- Ashburner, M. 1990. *Drosophila, a laboratory handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Bender, W., P. Spierer, and D.S. Hogness. 1983. Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the *bithorax* complex in *Drosophila melanogaster*. *J. Mol. Biol.* **168**: 17–33.
- Benezra, R., R.L. Davis, D. Lockshon, D.L. Turner, and H. Weintraub. 1990. The protein Id: A negative regulator of helix-loop-helix DNA binding proteins. *Cell* **61**: 49–59.
- Benson, M. and V. Pirrotta. 1988. The *Drosophila zeste* protein binds cooperatively to sites in many gene regulatory regions: Implications for transvection and gene regulation. *EMBO J.* **7**: 3907–3915.
- Bickel, S. and V. Pirrotta. 1990. Self-association of the *Drosophila zeste* protein is responsible for transvection effects. *EMBO J.* **9**: 2959–2967.
- Dreesen, T.D., D.H. Johnson, and S. Henikoff. 1988. The brown protein of *Drosophila melanogaster* is similar to the white protein and to components of active transport complexes. *Mol. Cell. Biol.* **8**: 5206–5215.
- Ellis, H.M., D.R. Spann, and J.W. Posakony. 1990. *extramacrochaetae*, A negative regulator of sensory organ development in *Drosophila*, defines a new class of helix-loop-helix proteins. *Cell* **61**: 27–38.
- Ephrussi, B. and E. Sutton. 1944. A reconsideration of the mechanism of position effect. *Proc. Natl. Acad. Sci.* **30**: 183–197.
- Farmer, J.L. and D.J. Fairbanks. 1986. Interaction of the *bw* and *w* loci in *D. melanogaster*. *Drosophila Inf. Svc.* **63**: 50–51.

- Frankham, R. 1988. Molecular hypotheses for position-effect variegation: Anti-sense transcription and promoter occlusion. *J. Theor. Biol.* **135**: 85–107.
- Garrell, J. and J. Modolell. 1990. The *Drosophila extramacrochaetae* locus, an antagonist of proneural genes that, like these genes, encodes a helix-loop-helix protein. *Cell* **61**: 39–48.
- Gubb, D., M. Ashburner, J. Roote, and T. Davis. 1990. A novel transvection phenomenon affecting the *white* gene of *Drosophila melanogaster*. *Genetics* **126**: 167–176.
- Henikoff, S. 1979. Position effects and variegation enhancers in an autosomal region of *Drosophila melanogaster*. *Genetics* **93**: 105–115.
- . 1981. Position-effect variegation and chromosome structure of a heat shock puff in *Drosophila*. *Chromosoma (Berl)* **83**: 381–393.
- . 1990. Position-effect variegation after 60 years. *Trends Genet.* **6**: 422–426.
- Henikoff, S. and T.D. Dreesen. 1989. Trans-inactivation of the *Drosophila brown* gene: Evidence for transcriptional repression and somatic pairing dependence. *Proc. Natl. Acad. Sci.* **86**: 6704–6708.
- Judd, B.H. 1988. Transvection: Allelic cross talk. *Cell* **53**: 841–843.
- Karpen, G. and A.C. Spradling. 1990. Reduced DNA polytenization of a minichromosome region undergoing position-effect variegation in *Drosophila*. *Cell* **63**: 97–107.
- Laird, C.D. 1990. Proposed genetic basis of Huntington's disease. *Trends Genet.* **6**: 242–247.
- Laurie-Ahlberg, C.C. and L.F. Stam. 1987. Use of *P*-element-mediated transformation to identify the molecular basis of naturally occurring variants affecting *Adh* expression in *Drosophila melanogaster*. *Genetics* **115**: 129–140.
- Lewis, E.B. 1950. The phenomenon of position effect. *Adv. Genet.* **3**: 73–115.
- Lindsley, D.L. and E.H. Grell. 1968. Genetic variations of *Drosophila melanogaster*. Carnegie Institute, Washington, D.C.
- Misner, D. and G.R. Rubin. 1987. Analysis of the promoter of the *ninaE* opsin gene in *Drosophila melanogaster*. *Genetics* **116**: 565–578.
- Muller, H.J. 1932. Further studies on the nature and causes of gene mutations. *Proc. Intl. Congr. Genet.* **1**: 213–225.
- O'Donnell, J., R. Boswell, T. Reynolds, and W. Mackay. 1989. A cytogenetic analysis of the *Punch-tudor* region of Chromosome 2R in *Drosophila melanogaster*. *Genetics* **121**: 273–280.
- Robertson, H.M., C.R. Preston, R.W. Phillis, D.M. Johnson-Schlitz, W.K. Benz, and W.R. Engels. 1988. A stable genomic source of *P* element transposase in *Drosophila melanogaster*. *Genetics* **118**: 461–470.
- Rushlow, C.A., W. Bender, and A. Chovnick. 1984. Studies on the mechanism of heterochromatic position effect at the *rosy* locus of *Drosophila melanogaster*. *Genetics* **108**: 603–615.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Schultz, J. 1936. Variegation in *Drosophila* and the inert heterochromatic regions. *Proc. Natl. Acad. Sci.* **22**: 27–33.
- Simon, J.A., C.A. Sutton, R.B. Lobel, R.L. Glaser, and J.T. Lis. 1985. Determinants of heat shock-induced chromosome puffing. *Cell* **40**: 805–817.
- Slatkin, H.M. 1955. Position effects at the brown locus in *Drosophila melanogaster*. *Genetics* **40**: 5–23.
- Spofford, J.B. 1976. Position-effect variegation in *Drosophila*. In *Genetics and biology of Drosophila*, vol. 2a, pp. 955–1019. Academic Press, London.
- Spradling, A.C. 1986. *P* element-mediated transformation. In *Drosophila: A practical approach*. pp. 175–197. IRL Press, Oxford.
- Spradling, A.C. and G.M. Rubin. 1983. The effect of chromosomal position on the expression of the *Drosophila* xanthine dehydrogenase gene. *Cell* **34**: 47–57.
- Steller, H. and V. Pirrotta. 1985. A transposable *P* vector that confers selectable G418 resistance to *Drosophila* larvae. *EMBO J.* **4**: 167–171.
- Stern, C. and G. Heidenthal. 1944. Materials for the study of the position effect of normal and mutant genes. *Proc. Natl. Acad. Sci.* **30**: 197–205.
- Stern, C. and M. Kodani. 1955. Studies on the position effect on the cubitus interruptus locus of *Drosophila melanogaster*. *Genetics* **40**: 343–373.
- Sullivan, D.T. and M.C. Sullivan. 1975. Transport defects as the physiological basis for eye color mutants of *Drosophila melanogaster*. *Biochem. Genet.* **13**: 603–613.
- Wakimoto, B. and M. Hearn. 1990. The effects of chromosome rearrangements on the expression of heterochromatic genes in Chromosome 2L of *Drosophila melanogaster*. *Genetics* **125**: 141–151.
- Wilson, C., R.K. Pearson, H.J. Bellen, C.J. O'Kane, U. Grossniklaus, and W.J. Gehring. 1989. *P*-element-mediated enhancer detection: An efficient method for isolating and characterizing developmentally regulated genes in *Drosophila*. *Genes & Dev.* **3**: 1301–1313.
- Wu, C.-T. and M.L. Goldberg. 1989. The *Drosophila zeste* gene and transvection. *Trends Genet.* **5**: 189–194.
- Zachar, Z., C.H. Chapman, and P.M. Bingham. 1985. On the molecular basis of transvection effects and the regulation of transcription. *Cold Spring Harbor Symp. Quant. Biol.* **50**: 337–346.

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