

Modifiers of Terminal Deficiency-Associated Position Effect Variegation in *Drosophila*

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ABSTRACT

Terminal deletions of a *Drosophila* minichromosome (*Dp(1;f)1187*) dramatically increase the position effect variegation (PEV) of a *yellow*⁺ body-color gene located *in cis*. Such terminal deficiency-associated PEV (TDA-PEV) can be suppressed by the presence of a second minichromosome, a phenomenon termed “*trans*-suppression.” We performed a screen for mutations that modify TDA-PEV and *trans*-suppression. Seventy suppressors and enhancers of TDA-PEV were identified, but no modifiers of *trans*-suppression were recovered. Secondary analyses of the effects of these mutations on different PEV types identified 10 mutations that modify only TDA-PEV and 6 mutations that modify TDA-PEV and only one other type of PEV. One mutation, a new allele of *Su(var)3-9*, affects all forms of PEV, including silencing associated with the insertion of a transgene into telomeric regions (TPE). This *Su(var)3-9* allele is the first modifier of PEV to affect TPE and provides a unique link between different types of gene silencing in *Drosophila*. The remaining mutations affected multiple PEV types, indicating that general PEV modifiers impact TDA-PEV. Modifiers of TDA-PEV may identify proteins that play important roles in general heterochromatin biology, including proteins involved in telomere structure and function and the organization of chromosomes in the interphase nucleus.

THE organization of DNA in eukaryotic nuclei goes far beyond packaging into nucleosomes and chromosomes. Chromosomes in the metazoan interphase nucleus display two types of cytologically and functionally distinct chromatin: heterochromatin and euchromatin. Heterochromatin is late replicating, composed of highly and moderately repetitive sequences (satellite DNA and transposons), and is relatively gene poor. Conversely, euchromatin predominantly replicates in early to mid-S-phase, is composed mostly of unique sequences, and contains the vast majority of mutable genes (GATTI and PIMPINELLI 1992; WEILER and WAKIMOTO 1995; WALLRATH 1998; HENNIG 1999).

Cytological and genetic data suggest that the eukaryotic nucleus maintains a reproducible organization during interphase (BRIDGER and BICKMORE 1998; LAMOND and EARNSHAW 1998; LEITCH 2000). For example, interphase chromosomes can be organized into a “Rabl configuration,” in which telomeres and centromeres (which are constitutively heterochromatic) are clustered at opposite sides of the nuclear periphery, while the euchromatic portion of the genome is located predominantly in the nuclear lumen (RABL 1885; COMINGS

1980; MATHOG *et al.* 1984; HOCHSTRASSER *et al.* 1986; FUNABIKI *et al.* 1993). Even in nuclei in which the Rabl configuration is not observed, cytological studies show that telomeres associate with the nuclear lamina in a wide variety of organisms. Furthermore, in some tissues each chromosome, or specific parts of a chromosome, inhabits restricted, unique domains within interphase nuclei (BRIDGER and BICKMORE 1998; DIETZEL *et al.* 1998; VISSER *et al.* 1998; ZINK *et al.* 1998).

Genetic and cytological studies provide evidence that the structure of chromosomes and the location of genes along the chromosome, and perhaps within the interphase nucleus, can impact gene expression. One particularly well-studied example of the relationship between nuclear organization, chromosome structure, and gene expression is position effect variegation (PEV). PEV describes the clonal inactivation of a euchromatic gene that has been positioned close to or within heterochromatin (via chromosome aberration or transgene insertion) or silencing of heterochromatic genes that have been positioned in distal parts of chromosome arms (WEILER and WAKIMOTO 1995; WALLRATH 1998). PEV is sensitive to a large number of genetic modifiers [*Mod(var)s*], loci known as suppressors or enhancers of variegation [*Su(var)s* and *E(var)s*]. Genetic screens for *Drosophila Mod(var)s* suggest that >100 genes affect PEV associated with chromosome aberrations (GRIGLIATTI 1991; REUTER and SPIERER 1992).

In addition to being directly affected by chromosome structure, gene expression is also impacted by chromo-

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somal organization in the interphase nucleus. For example, translocation of the normally heterochromatic *light* (*lt*) gene to distal euchromatic regions results in *lt* variegation (WAKIMOTO and HEARN 1990). In addition, variegation of the euchromatic *brown* (*bw*) gene is enhanced by chromosome rearrangements that move the locus to more proximal positions within the euchromatin and is suppressed by rearrangements that move *bw* to more distal positions (TALBERT *et al.* 1994). Cytological studies indicate that *bw* variegation is correlated with increased associations of the gene with centric heterochromatin (CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996). Such genetic and cytological observations have led to models suggesting that heterochromatic and euchromatic "domains" exist within the nucleus and that appropriate positioning of a gene within the nucleus and relative to other chromosome regions is required for normal function (WAKIMOTO and HEARN 1990; KARPEN 1994; HENIKOFF 1997; BRIDGER and BICKMORE 1998; LAMOND and EARNSHAW 1998).

Genes located within subtelomeric regions of yeast (GOTTSCHLING *et al.* 1990; LUSTIG 1998; NIMMO *et al.* 1998) and *Drosophila* (LEVIS *et al.* 1985; KARPEN and SPRADLING 1992; WALLRATH and ELGIN 1995) frequently undergo silencing. In *Saccharomyces cerevisiae*, telomeres are clustered at the edge of the nucleus, and mutations in some genes (*e.g.*, *Hdf1* or *Hdf2*) interfere with telomere-induced silencing and telomere length, as well as telomere clustering and association with the nuclear periphery (BOULTON and JACKSON 1998; LAROCHE *et al.* 1998). *Drosophila* telomeres do not contain the tandem, simple repeats observed in most eukaryotes. Instead, *Drosophila* telomeres are composed of more complex repetitive DNAs, including the retrotransposons *TART* and *Het-A* (LEVIS *et al.* 1993; BIESSMANN and MASON 1997), and subtelomeric repeats known as telomere-associated sequences (TAS; PARDUE and DEBARYSHE 1999). Despite the unusual nature of telomeric DNA in *Drosophila*, genes inserted into subtelomeric regions are silenced (LEVIS *et al.* 1985; KARPEN and SPRADLING 1992; WALLRATH and ELGIN 1995). Strikingly, general *Mod(var)s* in *Drosophila* fail to alter the silencing associated with transgene insertion into telomeres (known as telomeric position effect, or TPE). Thus, despite their phenotypic similarity, different types of silencing in *Drosophila* can involve distinct components and mechanisms.

Despite the growing amount of evidence that telomere function, chromosome position, and nuclear organization play key roles in gene expression, surprisingly little is known about the components and mechanisms responsible for nuclear organization in multicellular organisms. In addition, while studies in mammals and yeasts have identified a number of telomeric proteins (COOPER 2000; MCEACHERN *et al.* 2000), very few proteins are known to be involved in *Drosophila* telomere function. Previous studies showed that the *yellow* (*y*) PEV

associated with minichromosome *Dp(1;f)1187* (*Dp1187*) is enhanced by deletions removing the distal portion of the minichromosome, even when the break is >100 kb distal to the *y* locus (TOWER *et al.* 1993; DONALDSON and KARPEN 1997). This terminal deficiency-associated PEV (TDA-PEV) is suppressed by the presence of a second minichromosome, a phenomenon termed *trans*-suppression. *Trans*-suppression of TDA-PEV is altered by structural changes in the *trans*-suppressing minichromosome, suggesting that TDA-PEV and *trans*-suppression involve chromosome nuclear organization and/or telomere structure and function (TOWER *et al.* 1993; DONALDSON and KARPEN 1997). Here, we report the results of a genetic screen for modifiers of TDA-PEV and *trans*-suppression. We have identified and characterized specific modifiers of TDA-PEV, as well as general *Mod(var)s*. This collection should help elucidate the components and mechanisms involved in heterochromatin biology, telomere function, and nuclear organization in *Drosophila* and their impact on the long-distance regulation of gene expression.

MATERIALS AND METHODS

Fly stocks and basic husbandry: Our standard genetic background is *y¹; ry⁵⁰⁶*. *X¹X* refers to *C(1)RM, y v*, which is composed of two X chromosomes attached to a single centromere (LINDSLEY and ZIMM 1992). Minichromosomes $\gamma 878$ and $\gamma 158$ have been previously described (LE *et al.* 1995; DONALDSON and KARPEN 1997). The *bw^d*; *st* stock was supplied by Dr. Steve Henikoff (TALBERT *et al.* 1994). *39C-3*, *39C-4*, *39C5*, and *39C-27* were supplied by Dr. Lori Wallrath (WALLRATH and ELGIN 1995). The *In(1)w^{mt}* chromosome was isolated by outcrossing an *In(1)w^{mt}*; *Su(var)2-10/SM1* stock (provided by Dr. Gunter Reuter) to our *y¹; ry⁵⁰⁶* strain, and the *In(1)w^{mt}*; *dp¹ bw¹* stock was from the Bloomington *Drosophila* Stock Center (stock no. 2880, in January 1997). All other mutations are as described in LINDSLEY and ZIMM (1992) or FlyBase (flybase.bio.indiana.edu). Flies were grown on standard corn flour, corn syrup media. All crosses were done at 21°, and all females used in crosses were virgins.

Mutagenesis and screen: A diagram of the screen is shown in Figure 2. Adult *y; ry; \gamma 158, ry⁺* males were collected 1–3 days posteclosion, aged for 2 days, and then treated with ethyl methanesulfonate (EMS). Mutagenesis was performed via overnight feeding of EMS in sugar water (12.5 mM EMS in 5% sucrose; ASHBURNER 1990). Green food coloring was added to the EMS solution to allow visual tracking of the EMS. EMS-mutagenized males were subsequently crossed *en masse* to $\gamma 878, y⁺$ -carrying females (25 males \times 50 females per bottle). Males were transferred to a new set of females after 2 days, while females were transferred to new bottles after 3 days to continue laying eggs. This procedure was repeated twice, such that each group of 25 EMS-treated males produced nine bottles of F₁ progeny (*i.e.*, males were crossed to three sets of females, and each set of females deposited eggs on three sets of bottles). $\gamma 878, y⁺$ -carrying females of two different types were used. Standard *X/X; \gamma 878, y⁺* females were used to isolate dominant autosomal modifier mutations, and *X¹X/Y; \gamma 878, y⁺* virgins were used to isolate either dominant autosomal modifiers or dominant or recessive X-linked modifiers.

Male progeny that carried $\gamma 878, y⁺$ (phenotypically *y⁺ ry⁻*) or both $\gamma 878, y⁺$ and $\gamma 158, ry⁺$ (phenotypically *y⁺ ry⁺*) were visually screened for increased or decreased levels of $\gamma 878 y⁺$

expression. A total of 49,554 F₁ males were screened from X/X mothers (from which we recovered dominant autosomal modifiers), and 25,420 F₁ males were screened from X^AX/Y mothers (from which X-linked and autosomal mutations were recovered). Male progeny with increased or decreased y⁺ expression were crossed to females (X^AX/Y females if the male had come from an X^AX/Y mother, X/X females if the male had come from an X/X mother) to determine if the phenotypes were heritable.

Mapping mutations to a chromosome and balancing mutations: Mutant male progeny from X^AX/Y mothers were crossed to X^AX/Y females. Mutations transmitted to all male progeny, and to no female progeny, were determined to be on the X chromosome; stocks of these mutations were maintained by crossing X^AX/Y females to their *mutant/Y*; $\gamma 878$, y⁺ brothers. Autosomal mutations were mapped to a chromosome and balanced by standard methods (ASHBURNER 1990), using *Sp/SMI*, *Cy*, or *ry/TM3*, *Sb* stocks (DONALDSON 2000). Five additional mutations were identified that were not on the X, second, or third chromosomes and are presumed to have been on the fourth chromosome.

Determining lethality level: Level of lethality was determined by standard lethality tests (ASHBURNER 1990). Five to seven females of genotype *Mutant/Balancer* were crossed to three to five males of genotype *Mutant/Balancer*. In the case of a completely viable mutation the number of expected non-balancer animals should equal one-half the number of balancer animals [NB = (0.5)Bal]. Mutations were classified on the basis of the number of nonbalancer animals obtained *vs.* expected: 0–5%, lethal; 6–50%, semilethal; and 51–75%, subviable (ASHBURNER 1990).

Complementation analysis: All 24-sec chromosome mutations were tested for lethal complementation with each other, as were most of the 21 third chromosome mutations (DONALDSON 2000). Five to seven females of genotype *Mutant A/Balancer* were crossed to three to five males of genotype *Mutant B/Balancer*. Mutations were classified as noncomplementing if the number of nonbalancer animals was 0–25% of expected. All complementation crosses were done reciprocally (*i.e.*, female *Mutant A/Balancer* crossed to male *Mutant B/Balancer* and male *Mutant A/Balancer* crossed to female *Mutant B/Balancer*). Crosses were classified as noncomplementing only if results from both crosses were consistent. At least 100 animals were counted for each cross, unless the outcome was obvious from 50 animals (*e.g.*, all the animals were *Cy*, indicating complete lack of complementation). Note that noncomplementation does not necessarily indicate mutations are in the same gene, since lethality may be due to second-site mutations rather than to the *Mod(var)* in question. In addition, mutations that complement each other may not necessarily be in different genes, since many of the mutations are not homozygous lethal and may not be expected to induce lethality in combination with each other. Thus, the estimate of the number of genes mutated is approximate.

Tests for modifier effects on w variegation: Tests for effects on w^{m4} PEV utilized two different lines, *In(1) w^{m4h} (w^{m4h})* and *In(1)w^{m4} y¹ w^{m4}; dp¹ bw¹ (w^{m4-2880})*, previously known as line number 2880 from the Bloomington Stock Center). Each mutation was tested for its effect on w^{m4} variegation at least twice; most mutations were tested using both alleles w^{m4h} and w^{m4-2880}. The 25 X-linked mutant chromosomes originally carried a wild-type copy of the *white (w)* gene, to be able to score for the presence of $\gamma 158$ (ry+). To test these mutations for effects on w PEV, we recombined the w¹¹⁸ allele onto the mutant X chromosomes (DONALDSON 2000).

To determine if mutations affect the PEV of a P element inserted into centric heterochromatin, all modifiers were tested for their effect on y¹ w¹¹⁸; *P[w⁺] 39C-3 (39C-3)*, a stock

in which a w⁺ P-element marker variegates due to its position within the 2L centric heterochromatin (WALLRATH and ELGIN 1995). A limited number of modifiers were also tested for their effect on y¹ w¹¹⁸; *P[w⁺] 39C-4 (39C-4)*, which contains a w⁺-marked P element inserted into another site in the 2L centric heterochromatin (WALLRATH and ELGIN 1995). We observed that the 39C-4 insertion is significantly less sensitive to genetic modification than is 39C-3; therefore, most secondary tests were performed only on 39C-3.

Modifiers were also tested to determine if they affect TPE. These tests utilized lines y¹ w¹¹⁸; *P[w⁺] 39C-5* (referred to as 39C-5) and y¹ w¹¹⁸; *P[w⁺] 39C-27* (referred to as 39C-27). The w⁺ genes of lines 39C-5 and 39C-27 variegate due to their insertion near the 2L and 2R telomeres, respectively (WALLRATH and ELGIN 1995). For the sake of simplicity, the w^{m4h}, w^{m4-2880}, 39C-3, 39C-4, 39C-5, and 39C-27 chromosomes are described collectively as “w^{var}.”

Two different crossing schemes were used to test the effects of the mutations on w^{var}, depending on whether the mutation was autosomal or X-linked. For autosomal mutations, five to seven w^{var} females were crossed to three to five *Mutant/Balancer* males. w^{var}, *Mutant* male progeny were visually scored for suppressed, enhanced, or unchanged variegation relative to control animals. Control crosses in which w^{var} females were crossed to +/*Balancer* males (where “+” represents the original, unmutagenized second or third chromosomes) were performed at the same time and on the same food, using virgins from the same collections. Phenotypes of +/*Balancer* progeny from test and control crosses were directly compared to ensure vial to vial consistency, and +/+ males (from the control vials) were compared to +/*Mutant* males (from the test vials) to ascertain the effect of the mutation on w^{var}. Each mutation was tested at least twice for its effect on all types of w^{var} PEV.

For X-linked mutations three to five y¹ w¹¹⁸ *mutant/Y* males were crossed to five to seven w^{var} females. y¹ w¹¹⁸/*Y* males were used for control crosses. Female y¹ w¹¹⁸ *mutant/w^{var}* progeny were scored for dominant suppressed, enhanced, or unchanged w⁺ variegation relative to y¹ w¹¹⁸/*w^{var}* progeny from control crosses.

Tests for modifier effects on bw^D position effect variegation: The *bw* locus is required to produce the pteridine (bright red) eye pigments (LINDSLEY and ZIMM 1992). The expression of pteridine eye pigments is much more easily seen in the absence of ommochrome (brown) pigments, which require the function of the *cinnabar (cn)* and *scarlet (st)* genes. We assessed the level of *bw* expression in a *cn* or *st* background so small changes in *bw* expression could be easily observed. As was described for w^{var} tests (above), control crosses were undertaken in parallel using the original, unmutagenized chromosome.

X-linked modifier effects on bw^D: y¹ *Mutant/FM7* females were crossed to *FM7/Y*; *bw^D/+*; *st/ry* males. *Mutant/FM7*; *bw^D/+*; *st/ry* progeny were crossed to *bw^D*; *st* males. Male *Mutant/Y*; *bw^D/+*; *st/st* progeny were scored for suppressed, enhanced, or unchanged bw^D variegation relative to control animals.

Second chromosome modifier effects on bw^D: *Mutant/SMI*, *Cy*; *ry* males were crossed to *bw^D*; *st* females. Male *Mutant/bw^D*; *st* progeny were backcrossed to *bw^D*; *st* females. *Mutant/bw^D*; *st* progeny were scored for bw^D PEV phenotype relative to control animals.

Third chromosome modifier effects on bw^D: *Mutant/TM3*, *Stubble (Sb)* females were crossed to *bw^D cn/bw*; +/*TM6B* males. Male *bw^D cn/+*; *Mutant/TM6B*, *Tubby (Tb)* progeny (selected as Sb+, Tb) were crossed to *cn*; + females. Modification of bw^D PEV was scored in *cn/cn bw^D*; +/*Mutant* progeny (selected as Tb+ Hu+).

Tests for modifier effects on a variegating yellow⁺ P-element insertion: To distinguish true modifiers of TDA-PEV from general modifiers of y PEV or y expression, we examined the

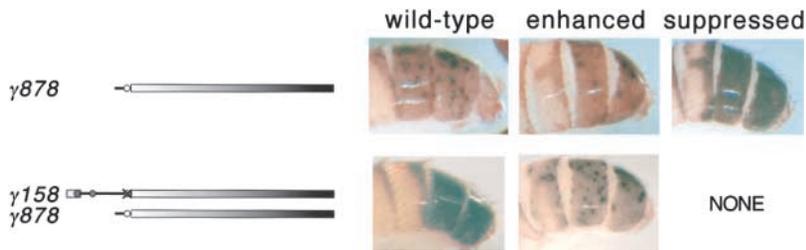


FIGURE 1.—Terminal deficiency-associated *yellow* PEV and *trans*-suppression phenotypes associated with *Dp* 8-23 minichromosome derivatives. $\gamma 878$, y^+ and $\gamma 158$, γ^+ are γ -irradiation derivatives of *Dp* 8-23 (LE *et al.* 1995). Structures include centric heterochromatin (shaded box), euchromatin (horizontal solid line), *yellow* locus (open circle), *rosy*⁺ P elements (solid circles), and subtelomeric heterochromatin (shaded box). *yellow* variegation phenotype is shown for adult male abdomens.

effect of TDA-specific modifiers on a y^+ P element inserted in the centric heterochromatin of the third chromosome (line B79; YAN *et al.* 2002). Experiments to test the effects of autosomal modifiers on B79 PEV were identical to those used to test mutant effects of w^{var} , except that the B79 chromosome was used instead of the w^{var} chromosomes. For X-linked modifiers, $X^{\wedge}X y v/Y$ virgins carrying the B79 chromosome were crossed to males that carried the mutation. Male progeny were scored for changes in B79 y^+ expression relative to progeny from control crosses.

Sequence analysis of *Su(var)3-9* and *Su(var)2-5* genes: Genetic data suggested that mutation 1699 could be a new allele of *Su(var)3-9* and that mutations 1009, 1097, 1207, and 1545 represented new alleles of *Su(var)2-5* (see RESULTS). To confirm the allelism and identify the molecular lesions, PCR products from the mutant and the original, unmutagenized (y^+ ; γ^{506}) chromosomes were sequenced directly on an ABI 3700 automated sequencer in The Salk Institute DNA Sequencing Facility. Base pair and corresponding amino acid changes were identified by comparing control and mutant sequences, using Sequencher 3.0 (see RESULTS).

RESULTS

Isolation of modifiers of TDA-PEV and *trans*-suppression: Previous studies determined that terminal deficiencies of minichromosome *Dp* 8-23 significantly enhanced the variegation of the *yellow* (*y*) locus located on the minichromosome (*i.e.*, $\gamma 878$, y^+ ; Figure 1; TOWER *et al.* 1993; DONALDSON and KARPEN 1997). This TDA-PEV is suppressed by the presence of a second, y^- , minichromosome ($\gamma 158$, γ^+) *in trans*, a phenomenon termed *trans*-suppression (Figure 1; DONALDSON and KARPEN 1997).

The consistency of the $\gamma 878$, y^+ *yellow* PEV phenotype allows for sensitive identification of suppressors and enhancers of TDA-PEV (Figure 1). We mutagenized $\gamma 158$, γ^+ -carrying adult males with the chemical mutagen EMS (see MATERIALS AND METHODS) and crossed them to females carrying $\gamma 878$, y^+ (Figure 2). Potential modifiers of TDA-PEV were identified by screening male progeny that were phenotypically y^+ γ^- (therefore carrying only $\gamma 878$, y^+). Potential modifiers of *trans*-suppression were identified by screening male progeny that were phenotypically y^+ γ^+ (therefore carrying both $\gamma 878$, y^+ and $\gamma 158$, γ^+). A pilot screen was performed to examine the feasibility of the system for identifying modifiers of TDA-PEV and *trans*-suppression. Ten mutations that affect TDA-PEV were identified

(nos. 26, E69, E113, 224, E226, 177, 234, 242, 600, and E646) and were analyzed further (see below).

The success of the pilot screen encouraged us to undertake a larger screen, using an isogenic y^+ ; γ^{506} strain. Approximately 27,225 F₁ $\gamma 878$, y^+ -carrying males (62,017 chromosomes) were screened to identify 282 suppressors and 159 enhancers, of which 50 suppressors and 1 enhancer of TDA-PEV proved to be stable and heritable. Approximately 25,492 F₁ $\gamma 878$, y^+ / $\gamma 158$, γ^+ males (58,168 chromosomes) were screened to identify 360 mutations that reduced the y^+ expression of $\gamma 878$, y^+ / $\gamma 158$, γ^+ animals. From these original 360 F₁ mutants, nine lines were identified that carried heritable, mappable presumptive modifiers of *trans*-suppression.

In total, we obtained 60 *Mod(var)* mutations that affected TDA-PEV or *trans*-suppression from the 120,185 chromosomes (52,717 males) examined in the large-scale screen (0.05% per chromosome, Table 1). Twenty-five of the 60 modifier mutations came from 14,571 males produced by $X^{\wedge}X/Y$ -bearing mothers, allowing X-linked *Mod(var)* mutations to be recovered (see MATERIALS AND METHODS and Figure 2); 20 of these mutations are X-linked. Further analysis was performed on the 10 mutations identified in the pilot screen and on the 60 identified from the large-scale screen (see below).

Testing modifiers of *trans*-suppression for effects on TDA-PEV: Mutations specifically altering *trans*-suppression ($\gamma 878$, y^+ / $\gamma 158$, γ^+ animals) would not be expected to affect TDA-PEV ($\gamma 878$, y^+ animals). However, all nine mutations that reduced y^+ expression in $\gamma 878$, y^+ / $\gamma 158$, γ^+ animals also reduced *y* expression in $\gamma 878$, y^+ -only siblings. Therefore, no modifiers specifically affecting *trans*-suppression were recovered from the screen of 25,492 F₁ $\gamma 878$, y^+ / $\gamma 158$, γ^+ progeny, and these mutations are classified as TDA-PEV modifiers in subsequent studies.

Characterization of mutations that modify TDA-PEV: The 70 modifier mutations were mapped to chromosomes and balanced, using standard mapping and balancing procedures (see MATERIALS AND METHODS). Twenty-four mutations mapped to the second chromosome, 21 to the third chromosome, and 25 to the X chromosome (Table 2). Mutations on the second and third chromosomes were tested to determine if they were homozygous viable and were categorized as lethal, semilethal, subviable, or viable (see MATERIALS AND

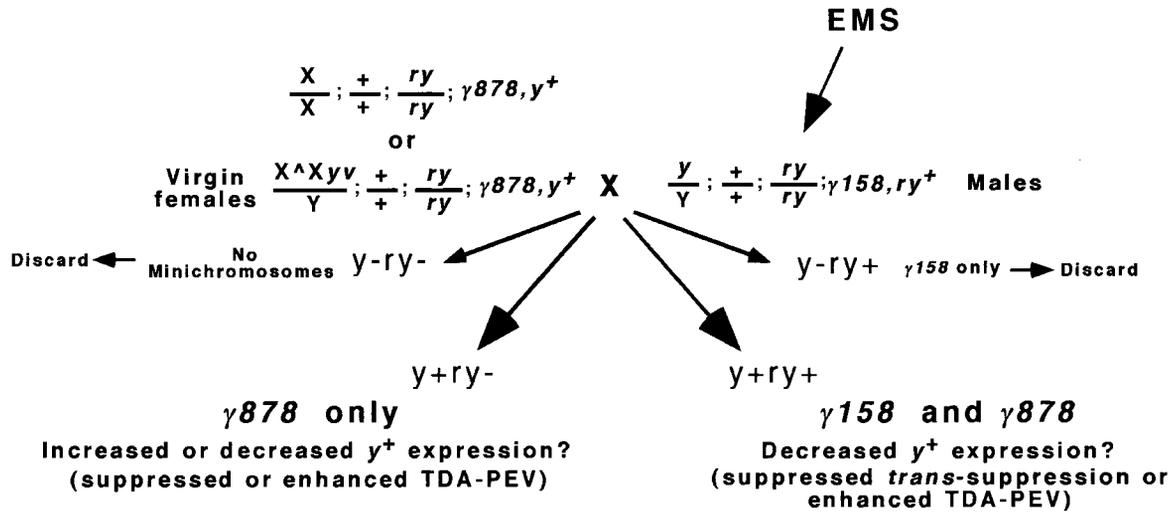


FIGURE 2.—Schematic describing the screen for modifiers of terminal deficiency-associated position effect variegation and *trans*-suppression. See text for details. $\gamma 158, ry^+$ males were EMS mutagenized and mated *en masse* to $\gamma 878, y^+$ -carrying virgin females. Phenotypically *yellow+* (and therefore $\gamma 878, y^+$ -carrying) male progeny were scored for modification of terminal deficiency-associated position effect variegation (TDA-PEV; phenotypically *ry-* males) or *trans*-suppression (phenotypically *ry+* males).

METHODS). Of 45 autosomal mutations, 18 are homozygous lethal, 15 are semilethal, 2 are subviable, and 10 are viable (Table 2).

All autosomal mutations in the collection were complementation tested to provide an estimate of how many

TABLE 1

Results of the large-scale screen for modifiers of TDA-PEV

F ₁ 878 males scored	
No. chromosomes	62,017
No. from X [∧] X/Y mothers	7,567
No. from X/X mothers	19,658
Total	27,225
F ₁ 878/158 males scored	
No. chromosomes	58,168
No. from X [∧] X/Y mothers	7,184
No. from X/X mothers	18,308
Total	25,492
No. potentially mutant F ₁ males	
dk 878 F ₁ males	282
lt 878 F ₁ males	159
dk 878/158 F ₁ males	0
lt 878/158 F ₁ males	360
No. heritable, stable mutations	
dk 878	50
lt 878	1
dk 878/158	NA
lt 878/158	9
Total F ₁ males scored	
Total chromosomes scored	120,185
<i>Su</i> (TDA-PEV)	50
<i>E</i> (TDA-PEV)	10
Frequency per chromosome	5.0×10^{-4}

dk, darker *y* phenotype (suppressed PEV); lt, lighter *y* phenotype (enhanced PEV); NA, not applicable. An additional 10 modifiers were isolated from the pilot screen; see text.

different loci were identified and to identify putative alleles at the same locus. Lethal complementation was examined (see MATERIALS AND METHODS), since the dominant PEV phenotype cannot be used to identify alleles at the same locus; unlinked, nonallelic PEV modifiers often interact additively or synergistically, which in most cases cannot be distinguished from noncomplementation of true alleles. The 24-sec chromosome mutations comprise 17 complementation groups: 1 group of four mutations (1009/1097/1207/1545), 1 group of three mutations (E1047/E1060/E1178), 2 groups of two mutations (1683/1227 and E1672/E646), and 13 single-mutation groups. The 21 third chromosome mutations comprise 20 complementation groups: 1 group of two mutations (234/1551) and 19 single-mutation groups. Note that it is possible that noncomplementation is due to second-site mutations on the chromosomes. Furthermore, complementation for lethality may not mean two mutations are nonallelic, since most of the mutations are not homozygous lethal, and hypomorphs may complement due to the presence of sufficient protein to ensure viability. Thus, the number of complementation groups is likely to be an overestimate of the number of different modifier loci isolated in the screen.

All 25 X-linked modifiers were identified in males; thus they are hemizygous viable; all 25 are also homozygous viable in females. All six X-linked enhancers of TDA-PEV are recessive, whereas 17 of the X-linked suppressors of TDA-PEV are dominant and 2 are recessive.

Secondary screening for effects on other types of PEV: Previous genetic screens suggest that >100 different loci modify PEV (GRIGLIATTI 1991; REUTER and SPIERER 1992). Mutations in at least two general *Mod(var)* genes suppress TDA-PEV: *Su(var)2-5* [which encodes Heterochromatin Protein 1 (HP1)]; JAMES *et*

TABLE 2
Secondary characterization of modifiers of TDA-PEV

Mutation line no.	Chromosome	Homozygous viability	Effect of mutations on PEV type					Complementation group
			<i>bw^D</i>	<i>w^{m4}</i>	<i>P(cen)</i>	<i>P(telo)</i>	<i>P(y⁺)</i>	
Group I								
<i>E1350</i>	X	NBD	—	—	—	—	ND	D
<i>1038</i>	2	Viable	—	—	—	—	—	
<i>E1047/E1060/E1178</i>	2	Lethal	—	—	—	—	—	
<i>1310</i>	2	Semilethal	—	—	—	—	—	
<i>1429</i>	2	Lethal	—	—	—	—	—	
<i>1657</i>	2	Lethal	—	—	—	—	—	
<i>1025</i>	3	Viable	—	—	—	—	—	
<i>1650</i>	3	Semilethal	—	—	—	—	—	
Group II								
<i>1699</i>	3	Viable	+	+	+	+	+	
Group III								
<i>26</i>	X	NBD	—	—	—	—	+	
<i>E226</i>	X	NBD	—	—	—	—	+	
<i>E69</i>	X	NBD	+	—	—	—	—	
<i>E113</i>	X	NBD	+	—	—	—	—	
<i>1309</i>	X	NBD	—	+	—	—	—	
<i>E1451</i>	X	NBD	—	—	+	—	—	
Group IV: Subgroup A								
<i>224</i>	X	NBD	+	+	+	—	—	B
<i>1474</i>	X	NBD	+	+	+	—	—	
<i>1328</i>	X	NBD	+ ^D	+	+	—	—	
<i>1356</i>	X	NBD	+ ^D	+	+	—	—	
<i>1367</i>	X	NBD	+	+	+	—	—	
<i>1444</i>	X	NBD	+	+	+	—	—	
<i>1447</i>	X	NBD	+	+	+	—	+	
<i>1474</i>	X	NBD	+	+	+	—	—	
<i>1485</i>	X	NBD	+ ^D	+	+	—	—	
<i>1539</i>	X	NBD	+	+	+	—	—	
<i>1009/1097 1207/1545</i>	2	Lethal	+	+	+	—	—	
<i>1126</i>	2	Semilethal	+	+	+	—	—	
<i>E1377</i>	2	Semilethal	+	+	+	—	—	
<i>1457</i>	2	Lethal	+	+	+	—	—	
<i>234/1551</i>	3	Lethal	+	+	+	—	—	
<i>1128</i>	3	Semilethal	+	+	+	—	+	
<i>1173</i>	3	Lethal	+	+	+	—	—	
<i>1181</i>	3	Subviable	+	+	+	—	+	
<i>1205</i>	3	Subviable	+	+	+	—	—	
<i>1260</i>	3	Semilethal	+	+	+	—	—	
<i>1602</i>	3	Semilethal	+	+	+	—	—	
Group IV: Subgroup B								
<i>1420</i>	X	NBD	—	+	+	—	—	C
<i>177</i>	2	Viable	—	+	+	—	—	
<i>E646/E1672</i>	2	Semilethal	—	+	+	—	—	
<i>1094</i>	2	Semilethal	—	+	+	—	—	
<i>1116</i>	2	Viable	—	+	+	—	—	E
<i>1227/1683</i>	2	Semilethal	—	+	+	—	—	
<i>242</i>	3	Lethal	—	+	+	—	+	
<i>1044</i>	3	Lethal	—	+	+	—	—	
<i>1144</i>	3	Viable	—	+	+	—	—	
<i>1200</i>	3	Lethal	—	+	+	—	—	
<i>1250</i>	3	Viable	—	+	+	—	—	
<i>1259</i>	3	Viable	—	+	+	—	—	
<i>1453</i>	3	Viable	—	+	+	—	+	
<i>1557</i>	3	Semilethal	—	+	+	—	+	
<i>1641</i>	3	Semilethal	—	+	+	—	—	
<i>1658</i>	3	Viable	—	+	+	—	—	

(continued)

TABLE 2
(Continued)

Mutation line no.	Chromosome	Homozygous viability	Effect of mutations on PEV type					Complementation group
			<i>bw^D</i>	<i>w^{m4}</i>	<i>P(cen)</i>	<i>P(telo)</i>	<i>P(y⁺)</i>	
Group VI: Subgroup C								
1294	X	NBD	+	+	—	—	—	
1317	X	NBD	+	+	—	—	—	
1322	X	NBD	+	+	—	—	—	
600	2	Lethal	+	+	—	—	—	
E1261	2	Semilethal	+	+	—	—	—	
1552	2	Lethal	+	+	—	—	—	
1535	X	NBD	ND	+	—	—	—	
Partially analyzed								
E1275	X	NBD	+	ND	ND	ND	—	
1515	X	NBD	—	ND	ND	ND	+	
1540	X	NBD	+	ND	ND	ND	+	

Mutations were separated into groups I–IV on the basis of their effect on PEV types *bw^D*, *w^{m4}*, *P(cen)*, *P(telo)*, and *P(y⁺)*. See text and MATERIALS AND METHODS for a description of PEV types and testing methods. Within each group and subgroup, mutations are listed in order of the chromosome on which they are found (X, second, or third) and then in numerical order on the basis of their line number. Mutations were tested for dominant effects on PEV, with the exception of X chromosome mutations tested for their recessive effects on *bw^D* and on *P(y⁺)*. Mutations that enhance TDA-PEV are denoted here by the presence of an uppercase “E” before the mutation’s number, e.g., E113. The remaining mutations, which are suppressors of TDA-PEV, are denoted only by their line number, e.g., 26.

Columns describe (from left to right) the mutation line number, the chromosome on which the mutation is located, homozygous viability, the effect of mutations on a given PEV type, and complementation group. “+” indicates the mutation had an effect on the type; “–” indicates the mutation had no effect. ND or blank, not done; NBD, not by definition (X-linked mutations were identified in males; all X-linked mutations are hemizygous and homozygous viable). A “+” in the *bw^D* column indicates the mutation has only a recessive effect, while “+^D” indicates the mutation affected *bw^D* PEV dominantly. *P(y⁺)* was tested only for recessive effects induced by X chromosome mutations. Mutations in the same complementation group are described on one line; for example, E1047/E1060/E1178 corresponds to three mutations that fail to complement each other for lethality and behave the same way in all other regards.

al. 1989; EISENBERG *et al.* 1990] and *Su(var)2-10/PIAS* (WUSTMANN *et al.* 1989; HARI *et al.* 2001). Therefore, we examined whether TDA-PEV modifiers affected TDA-PEV only or also impacted other types of PEV.

Mutations were characterized using five different PEV types, which we refer to as *w^{m4}*, *P(cen)*, *bw^D*, *P(telo)*, and *P(y⁺)* (MATERIALS AND METHODS). The structures of these chromosomes are shown in Figure 3, while the phenotypes for these lines are displayed in Figure 4 (top row, wild type). *w^{m4}* is a large inversion derivative of the X chromosome (Figure 3; TARTOF *et al.* 1984) that causes the *w* locus to be positioned very close to centric heterochromatin, inducing variegated expression (see MATERIALS AND METHODS). *w^{m4}* and *w^{m4}* alleles have been used in previous screens for modifiers of PEV (REUTER and WOLFF 1981; SINCLAIR *et al.* 1983; LOCKE *et al.* 1988; WUSTMANN *et al.* 1989; DORN *et al.* 1993).

Previous studies indicated that PEV can occur when a transgene is positioned within centric heterochromatin (REUTER and WOLFF 1981; WUSTMANN *et al.* 1989; DORN *et al.* 1993; WALLRATH and ELGIN 1995). While PEV induced by chromosome rearrangements (*i.e.*, *w^{m4}*) and *P*-element insertion are phenotypically similar, experiments to determine whether they are mechanistically similar have produced varied results (WALLRATH and

ELGIN 1995; SASS and HENIKOFF 1998). To address this issue, and to identify mutations that have differential effects on PEV due to inversion *vs.* *P*-element centric insertion, secondary testing was also applied to a *white⁺* *P* element inserted in centric heterochromatin [*P(cen)*; lines 39C-3 and 39C-4; WALLRATH and ELGIN 1995; see MATERIALS AND METHODS and Figure 3).

The third PEV type utilized for secondary screening was *bw^D*. The *bw^D* allele contains a large (~1.5 Mb) insertion of centric heterochromatin within the coding region of the *bw* locus (PLATERO *et al.* 1998). The insertion eliminates all *bw* expression from the *bw^D* chromosome. In addition, the *bw^D* chromosome causes PEV of the *bw⁺* allele on the homologous chromosome. Genetic and cytological data indicate that *bw^D* PEV is linked to an increased frequency of association of the *bw^D* allele and its paired *bw⁺* homolog with the centric heterochromatin (TALBERT *et al.* 1994; CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996). *bw^D* was used here as a secondary screen to identify mutations potentially involved in the nuclear organization or pairing of chromosomes.

Transgenes located in the subtelomeric regions of the chromosome [termed *P(telo)* in this study] are subject to position effect (LEVIS *et al.* 1985; WALLRATH and ELGIN 1995). Such TPE is phenotypically similar to PEV

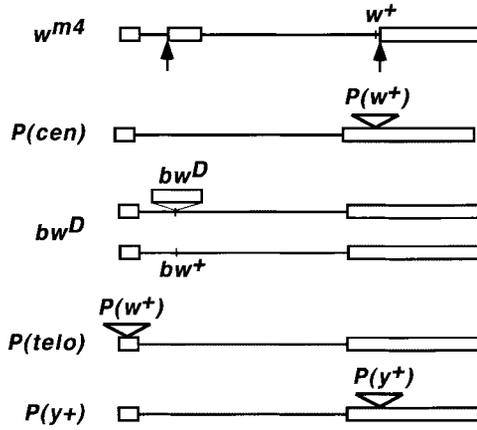


FIGURE 3.—Diagram of chromosome structures of PEV types used for 2° screening. Secondary screening of modifiers of TDA-PEV was undertaken using five different PEV types. Boxes, heterochromatin; thick solid lines, euchromatin; thin vertical lines, gene locations; inverted triangles, insertions. *w^{m4}* is a chromosomal inversion of the X chromosome that moved the *w⁺* locus close to heterochromatin, causing *w⁺* PEV. Arrows represent inversion breakpoints. *P(cen)* is an insertion of a *w⁺* P element into centric heterochromatin, causing PEV of the *w⁺* transgene. The *bw^D* allele contains a 1.5-Mb insertion of heterochromatin into the *bw* locus, which causes PEV of the *bw⁺* locus on the homologous chromosome. *P(telo)* is an insertion of a *w⁺* P element into subtelomeric heterochromatin, which induces PEV of the *w⁺* transgene. *P(y⁺)* is an insertion of a *y⁺* SUP_{or}-P element into centric heterochromatin of the third chromosome (B79), causing PEV of the *y⁺* transgene.

induced by other means. However, previous analyses failed to identify any *Su(var)*s or *E(var)*s that alter TPE (TALBERT *et al.* 1994; WALLRATH and ELGIN 1995; CRYDERMAN *et al.* 1999), indicating that the two phenomena are functionally distinct and are likely associated with different sets of proteins. Since TDA-PEV and TPE are both thought to be associated with telomere behavior, a mutation altering both TDA-PEV and TPE may identify a gene involved in telomere function. We used the 39C-5 and 39C-27 telomeric insertions of a *w⁺*-marked P ele-

ment to analyze effects of the modifier mutations on TPE (see MATERIALS AND METHODS; WALLRATH and ELGIN 1995).

To identify mutations specifically affecting TDA-PEV (and not just *y* PEV or *y* expression), 26 mutations were tested for their effect on a strain containing a variegating *y⁺* P element [referred to here as *P(y⁺)*] that is inserted in the centric heterochromatin of chromosome 3 (DOBIE *et al.* 2001; YAN *et al.* 2002).

Secondary screens identify four major groups of mutations: Sixty-seven of 70 TDA-PEV *Mod(var)* mutations were determined to enhance, suppress, or have no effect on each type of PEV, by direct comparisons with control animals that lacked the mutations (see MATERIALS AND METHODS). The three remaining mutations are all X-linked and for technical reasons could not be recombined onto the *y w* chromosome [a necessity for testing the effect of the mutations on *w^{m4}*, *P(cen)*, and *P(telo)* variegation; see MATERIALS AND METHODS].

The results of the secondary screening allowed us to place the mutations into one of four major groups, distinguished by their effects on the different PEV types (Table 2). Group I consists of mutations that affect only TDA-PEV. Group II contains a single mutation that has an effect on all types of PEV tested, including telomeric PEV. Group III includes mutations that affect TDA-PEV and only one other type of PEV. Group IV contains mutations that behave like classic modifiers of PEV; these mutations affect multiple variegating types, but not *P(telo)*. Images of characteristic phenotypes are displayed in Figure 4. Our data indicate that all members of a given complementation group behave similarly with respect to lethality and effect on different PEV types. Thus, subsequent details regarding the different mutation groups include the number of complementation groups in addition to, or instead of, the number of mutations showing a given phenotype.

Ten mutations affect only TDA-PEV: Of the 67 mutations (comprising at most 59 complementation groups)

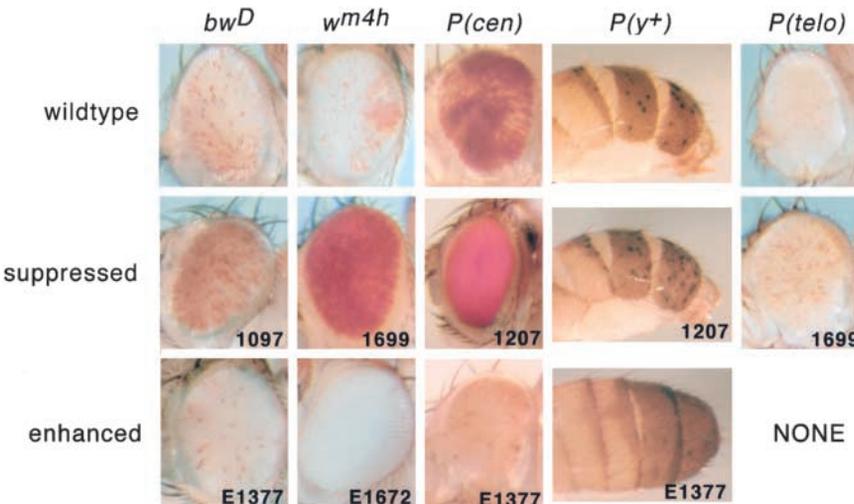


FIGURE 4.—Representative phenotypes of PEV types used for 2° screening. Phenotypes are shown for wild-type, suppressed, and enhanced PEV. Images shown are typical of observed phenotypes.

that have been fully characterized, 10 (8 complementation groups) affect only TDA-PEV and are placed in group I (Table 2). Mutations specific to TDA-PEV were found on the X, second, and third chromosomes and consist of both suppressors and enhancers of TDA-PEV. Three of the second chromosome mutations, all enhancers of TDA-PEV (*E1047*, *E1060*, and *E1178*), are homozygous lethal and comprise a single complementation group. Of the 4 remaining second chromosome group I mutations, 1 is homozygous viable (*1038*), 1 is semilethal (*1310*), and 2 are homozygous lethal (*1429* and *1657*). Of the mutations on the third chromosome, 1, *1025*, is homozygous viable, while the other, *1650*, is homozygous semilethal. The X-linked mutation *E1350* is homozygous viable; *E1350* has not been tested for its effect on $P(y^+)$ and thus could eventually be characterized as a group III mutation.

One novel modifier of TDA-PEV also affects telomeric position effect: Group II consists of a single mutation, *1699*, which suppresses TPE of both $P(telo)$ lines tested. This homozygous viable third chromosome mutation also suppresses all the other types of PEV examined in the secondary tests and is the first example of a general modifier of PEV that can affect TPE (TALBERT *et al.* 1994; WALLRATH and ELGIN 1995; CRYDERMAN *et al.* 1999).

Analysis of *1699* provided clues regarding its identity. For example, *1699* has a particularly strong effect on w^{m^4} , increasing w expression to nearly wild-type levels (data not shown). Recombination mapping of *1699* localized it to the genetic map region 3-56, very close to the location of a previously identified, extremely strong modifier of PEV, $Su(var)3-9$ (TSCHERSCH *et al.* 1994). Sequence analysis of *1699* (see MATERIALS AND METHODS) identified a mutation in the $Su(var)3-9$ gene, a T to A change in the first position of the codon encoding amino acid 456. This cysteine to serine change is in a residue that is completely conserved among $Su(var)3-9$ homologs, including *clr4* in *Schizosaccharomyces pombe* (IVANOVA *et al.* 1998), *suw39h1* and *suw39h2* in the mouse (AAGAARD *et al.* 1999; O'CARROLL *et al.* 2000), and SUV39H1 in humans (AAGAARD *et al.* 1999). These proteins have been demonstrated to encode a histone methyltransferase (see DISCUSSION).

Five mutations affect TDA-PEV and only one additional type of PEV: The six group III mutations have effects on TDA-PEV and only one other type of PEV. Lines *E69* and *E113* affect only TDA-PEV and bw^D PEV. These mutations are the first identified modifiers of bw^D PEV that are not also general modifiers of PEV. *E69* has a particularly unusual phenotype: although it enhances TDA-PEV, it suppresses bw^D PEV. Such differential modification of PEV types is a unique behavior for a PEV modifier; previously described mutations act either as suppressors or as enhancers of PEV in general. Line *1309* affects only TDA-PEV and w^{m^4} , while *E1451* affects only TDA-PEV and $P(cen)$. Finally, *26* and *E226* affect

only TDA-PEV and the y^+ centric P insertion; these mutations may affect y^+ expression, rather than PEV *per se*. Strikingly, all six mutations in group III are on the X chromosome. These mutations comprise a unique group that may provide insight into the mechanisms of and overlap between different types of PEV.

Mutations affecting position effect variegation in general: The remaining 50 mutations affect at least two PEV types in addition to TDA-PEV, indicating that they play a general role in position effect variegation (see Table 2). These 50 mutations comprise 44 complementation groups; 41 are suppressors and 3 are enhancers. In every case TDA-PEV suppressors also suppressed the other types of PEV, and enhancers of TDA-PEV behaved only as enhancers of other types of PEV. Group IV mutations are subdivided into subgroups A, B, and C on the basis of their effects on bw^D , w^{m^4} , and $P(cen)$ PEV. Mutations in subgroup A affect bw^D , w^{m^4} , and $P(cen)$, mutations in subgroup B fail to affect bw^D but do modify w^{m^4} and $P(cen)$, and mutations in subgroup C affect bw^D and w^{m^4} but fail to affect $P(cen)$. No mutations that modified bw^D and $P(cen)$ and did not affect w^{m^4} were identified.

Of the 44 group IV complementation groups, 21 are in subgroup A (Table 2), including 20 suppressors and 1 enhancer. Ten of these mutations are located on the X chromosome, 4 are on the second, and 7 are on the third. None of the autosomal subgroup A mutations are homozygous viable. Of particular interest is the single enhancer in subgroup A, *E1377*. Previous experiments have suggested that enhancers of w^{m^4} variegation do not affect bw^D PEV (SASS and HENIKOFF 1998). Indeed, no genic enhancers of bw^D PEV have been identified until now; *E1377* and another enhancer, *E1261* (in subgroup C), provide intriguing exceptions to this observation, as do mutations *E69* and *E113* (in group III, discussed previously).

Subgroup A also includes one complementation group that has four mutations (*1009*, *1097*, *1207*, and *1545*). Analysis of mitotic chromosomes from *1207/1207* and *1009/1009* homozygous larvae (H. LE, K. DONALDSON and G. KARPEN, unpublished results) demonstrated that these mutations exhibit telomere fusions, which is similar to the behavior of $Su(var)2-5$ (HP1) alleles (FANTI *et al.* 1998). Complementation analysis indicated that *1009*, *1097*, *1207*, and *1545* are all new alleles of $Su(var)2-5$, and sequence analyses confirmed that all four mutations represent lesions in the $Su(var)2-5$ locus. *1009* contains an A to T change at the first position of the codon for amino acid 46, resulting in a premature stop codon and presumed truncation of the protein; *1207* contains a C to T change at the first position of the codon for amino acid 69, leading to a premature stop codon and presumed truncation of the protein; *1097* contains an A to T change at the first position of the codon encoding amino acid 53, causing an asparagine to tyrosine change; *1545* has two mutations within the $Su(var)2-5$ locus, one a G to A change

in the first position of the codon for amino acid 27, causing a glutamic acid to lysine change, and the other an A to G change in the first position of the codon for amino acid 183, causing an asparagine to aspartic acid change.

Sixteen complementation groups comprise subgroup B. These mutations affect TDA-PEV, w^{m4} , and $P(cen)$ but have no effect on bw^D . Fifteen of these mutations are suppressors of PEV; 1 is on the X, 4 are on the second, and the remaining 10 are on the third. The single enhancer in subgroup B is on the second chromosome; this complementation group is composed of two mutations, *E646* and *E1672*. Whereas none of the subgroup A autosomal mutations were viable as homozygotes, nearly one-half (7 out of 15) of the subgroup B autosomal mutations are homozygous viable.

The remaining seven mutations comprise subgroup C. These six suppressors and one enhancer affect TDA-PEV, bw^D , and w^{m4} but do not affect $P(cen)$. Four of these suppressors are on the X chromosome, and the two second chromosome suppressors are both homozygous lethal, as is the single enhancer in this subgroup, *E1261*. Mutation *I535* has not been tested for its effect on bw^D and thus could be a member of group III.

DISCUSSION

Very few proteins involved in chromosome nuclear organization and telomere structure and function in *Drosophila* have been identified. Previous studies have described and characterized a novel form of position effect variegation associated with terminal deficiencies that position new chromosome ends distal to the *yellow* gene (TOWER *et al.* 1993; DONALDSON and KARPEN 1997). This TDA-PEV is suppressed by the presence of a second minichromosome *in trans*, a phenomenon termed *trans*-suppression (DONALDSON and KARPEN 1997). Previous data suggested that TDA-PEV and *trans*-suppression involve chromosome pairing, nuclear organization, and telomere structure and function (DONALDSON and KARPEN 1997).

Here, we describe the results of a screen for modifiers of TDA-PEV and *trans*-suppression. Seventy modifiers of TDA-PEV were identified, corresponding to at most 62 complementation groups. Secondary analysis of the mutations identified four classes of mutations, including mutations that affect only TDA-PEV, or TDA-PEV and a limited number of PEV types, as well as general PEV modifiers. In addition, we isolated a suppressor mutation (*I699*), corresponding to a new allele of *Su(var)3-9*, which is the first identified modifier of PEV to also affect TPE. We predict that the loci identified in this screen will help elucidate the relationship between chromosome nuclear organization, telomere structure and function, and gene expression.

Modifiers of TDA-PEV may provide a unique system for the study of eukaryotic nuclear organization: Ge-

netic and cytological observations have led to models suggesting that appropriate positioning of genes and chromosomes within interphase nuclei is required for normal expression (WAKIMOTO and HEARN 1990; KARPEN 1994; HENIKOFF 1997; BRIDGER and BICKMORE 1998; LAMOND and EARNSHAW 1998). In *S. cerevisiae*, telomeres are clustered at the edge of the nucleus, and mutations that interfere with telomere-induced silencing and telomere length also affect telomere clustering and association with the nuclear periphery (BOULTON and JACKSON 1998; LAROCHE *et al.* 1998). Similarly, in *S. pombe*, silenced chromatin is formed near the mating-type loci, telomeres, and centromeres. Mutations in some proteins alleviate silencing at all three loci (*e.g.*, *Swi6*; EKWALL *et al.* 1995), while other mutations affect only one type of silencing (*e.g.*, *Mis6*; SAITOH *et al.* 1997). Thus, studies of unicellular eukaryotes suggest that different types of silencing are regulated by different proteins. Such silencing may involve associations between genes and specific parts of the nucleus, reflecting the possible presence of distinct heterochromatic structural "domains."

Similar conclusions can be gleaned from studies of gene silencing in higher eukaryotes. In many cell types, heterochromatic telomere and centromere regions are associated with the nuclear lamina and are clustered at opposite sides of the nucleus, while the euchromatin is located predominantly in the nuclear lumen (RABL 1885; COMINGS 1980; MATHOG *et al.* 1984; HOCHSTRASSER *et al.* 1986; FUNABIKI *et al.* 1993). Chromosomes in mammals have also been demonstrated to occupy distinct and reproducible domains in interphase nuclei (BRIDGER and BICKMORE 1998; DIETZEL *et al.* 1998; VISSE *et al.* 1998; ZINK *et al.* 1998). Gene repression in *Drosophila* can be associated with the location of a gene at telomeres, at pericentric heterochromatin, and in association with Polycomb-group (Pc-G) proteins. Several factors have been found to affect only one or two of these types of silencing, suggesting there may be functionally separable silencing regions in *Drosophila*, as in yeasts. For example, analyses of general PEV modifiers failed to identify any that affected telomeric position effect (WALLRATH and ELGIN 1995; CRYDERMAN *et al.* 1999).

Secondary analyses of the TDA-PEV modifiers described here provide additional insight into the relationships among different types of PEV. The classification of these mutations into different groups on the basis of their spectrum of modification effects suggests that distinct components and mechanisms are responsible for different forms of silencing in *Drosophila*. The majority of mutations impacted w^{m4} , bw^D , and the PEV associated with centric heterochromatin *P*-element insertions (group IV, subgroup A), suggesting that many components are common to different types of PEV. However, only one mutation (*I699*, group II) affected all types of PEV tested, including TPE, suggesting that the *Su-*

(*var*)3-9 protein may be a component of most or all types of silencing in *Drosophila*.

Most importantly, we have identified mutations that alter a limited number of PEV types (groups III and IV, subgroups B and C) or that affect only TDA-PEV (group I). These data are consistent with the existence of multiple silencing mechanisms and suggest that the different types of silencing are physically and/or functionally distinct from each other, due perhaps to the presence or absence of specific proteins, specific protein domains, or associations with distinct nuclear domains.

Mutations that modify only TDA-PEV may help dissect telomere structure and function in *Drosophila*: *Drosophila* telomeres do not contain "canonical" telomerase-produced short tandem repeats (PARDUE and DEBARYSHE 1999; see Introduction). Nevertheless, telomeres in *Drosophila* perform the same basic functions as telomeres in other organisms (DERNBURG *et al.* 1995; COOPER 2000); they counter terminal sequence loss due to replication of a linear molecule, are associated with the nuclear envelope (HARI *et al.* 2001), prevent chromosome ends from fusing (FANTI *et al.* 1998), and identify the end of the chromosome as normal and not a double-stranded break that must be repaired (MCEachern *et al.* 2000). Terminal sequence loss by incomplete DNA replication is counterbalanced by rare *TART* and *Het-A* retroposon additions. However, it is obvious that the remaining functions of telomeres occur independently of *TART* and *Het-A* elements, since fully functional telomeres in *Drosophila* have been identified that have no *TART* or *Het-A* sequence near the ends (MASON *et al.* 1984; LEVIS 1989; BIESSMANN *et al.* 1990). These terminal deficiency chromosomes appear to have completely normal telomere function. They do not cause double-stranded break-dependent cell cycle arrest and show no evidence of chromosome end-to-end fusion (MASON *et al.* 1984; LEVIS 1989; BIESSMANN *et al.* 1990). *Drosophila* telomeres are also associated with at least one protein known to play a role in telomere function, HP1 (FANTI *et al.* 1998). These observations suggest that *Drosophila* telomeres are not defined by primary DNA sequence, but by some other factor, such as a specific chromatin structure, the presence of specific proteins, or some other epigenetic mark (BIESSMANN *et al.* 1990; AHMAD and GOLIC 1999). Indeed, in other organisms there is mounting evidence that although the telomerase enzyme is responsible for maintaining the length of telomeres, equally important proteins are playing critical roles in telomere functions, including telomere clustering, interaction with the nuclear envelope, and prevention of telomere fusion (VAN STEENSEL *et al.* 1998; COOPER 2000; THAM and ZAKIAN 2000). One particularly telling piece of data is that mice lacking telomerase are viable and appear normal for several generations (BLASCO *et al.* 1997; RUDOLPH *et al.* 1999), implying that other components can still act to promote telomere

functions in the absence of telomerase-mediated maintenance of telomeric DNA sequence.

Since TDA-PEV involves placement of a chromosome end closer to the *yellow* gene, further study of the genes that modify TDA-PEV is likely to improve our understanding of telomere biology and nuclear organization in *Drosophila*. Indeed, one *Mod(var)* [*Su(var)2-10*] encodes a protein [protein inactivator of activated STAT (PIAS)] that is localized to the nuclear lamina and telomeres (HARI *et al.* 2001). *Su(var)2-10* mutations suppress TDA-PEV and alter telomere-telomere and telomere-lamina associations. These results support the hypothesis that the SU(VAR)2-10 protein coordinates telomere functions and is required for normal nuclear organization in interphase. They also suggest that other modifiers of TDA-PEV are likely to play roles in telomere structure and function.

Secondary tests were performed on 67 TDA-PEV modifier mutations, using various PEV types (Figure 2). Of greatest interest are 10 mutations, corresponding to at most eight complementation groups, which affected only TDA-PEV and no other type of PEV (group I, Table 2). Three of the mutations (*E1047*, *E1060*, and *E1178*) fail to complement each other for lethality, suggesting that they are all mutations in the same locus. Group I mutations are not general PEV modifiers, suggesting that TDA-PEV is a specialized type of PEV that involves novel factors in addition to general modifiers of PEV.

If the group I mutations alter telomere function, then why do they have no effect on TPE? It is possible that TDA-PEV and TPE are associated with different telomere regions, functions, or proteins. Alternatively, TPE may occur independently of telomere function. In most cases of TPE, the variegation occurs after insertion of a transgene into the TAS elements, large tandemly repeated arrays that are found just proximal of the *Het-A* and *TART* arrays at the ends of *Drosophila* chromosomes (KARPEN and SPRADLING 1992; KURENOVA *et al.* 1998). Complementary results suggest that TPE is induced by the TAS elements rather than telomeric location. First, TAS elements can act as boundary elements when they are located in more proximal positions along the chromosome arm (KURENOVA *et al.* 1998). Thus, TAS elements affect gene expression independent of their telomeric position. Second, terminal deficiencies broken just distal to the inserted transgene, which eliminate the distal TAS repeats, result in reversion of TPE (LEVIS 1989; TOWER *et al.* 1993). These results suggest that flanking TAS elements are responsible for TPE, rather than the presence of a chromosome end. Finally, at least two proteins with demonstrated roles in telomere behavior [*Su(var)2-10*/PIAS and *Su(var)2-5*/HP1; FANTI *et al.* 1998; HARI *et al.* 2001] affect TDA-PEV and not TPE. Thus, it is likely that mutations that affect only TDA-PEV will identify proteins involved in the maintenance of chromosome ends or their nuclear positions and other chromosomal functions.

Mutation 1699, a general modifier of PEV, also affects telomeric position effect: Only 1 of the 67 mutations that affect TDA-PEV, *1699*, modified all types of PEV tested, including TPE. This result is surprising; only a few mutations are known to modify TPE, and *1699* represents the first example of a mutation that affects both PEV and TPE (TALBERT *et al.* 1994; WALLRATH and ELGIN 1995; KURENOVA *et al.* 1998). Recombination mapping, phenotypic analyses, and sequence analyses demonstrated that *1699* represents a new allele of *Su(var)3-9* (TSCHERSCH *et al.* 1994). SU(VAR)3-9 homologs are found in a wide range of organisms, including *S. pombe* (IVANOVA *et al.* 1998), mice, and humans (AAGAARD *et al.* 1999; O'CARROLL *et al.* 2000). SU(VAR)3-9 homologs encode methyltransferases that modify lysine 9 of histone H3 and have been demonstrated to play roles in the recruitment of HP1 to heterochromatin and in chromosome segregation and mitotic progression (EKWALL *et al.* 1996, 1999; AAGAARD *et al.* 1999; REA *et al.* 2000; BANNISTER *et al.* 2001; LACHNER *et al.* 2001). *1699* contains a mutation in a cysteine residue that is conserved throughout the SU(VAR)3-9 homologs, part of a cysteine-rich region required for histone H3 lysine 9 methyltransferase activity (REA *et al.* 2000). We conclude that SU(VAR)3-9 may also play a role in telomere function and nuclear organization and that at least one protein functions in both TPE and PEV.

Some mutations affect TDA-PEV and only one other type of PEV: Most modifiers of PEV act on multiple types of PEV, suggesting they represent mutations in genes involved in general heterochromatin biology and common aspects of gene silencing. However, secondary analysis of TDA-PEV modifiers also identified mutations that affect only a subset of PEV types. Two enhancer mutations (*E69* and *E113*) affected only TDA-PEV and *bw^p*. Genetic and cytological studies indicate that *bw^p* PEV is associated with mitotic pairing of homologous chromosomes and alterations in the associations of the *bw⁺* chromosome with centric heterochromatin and likely reflects the importance of nuclear organization to gene expression (TALBERT *et al.* 1994; CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996). Thus, mutations that act specifically on TDA-PEV and *bw^p* may identify proteins that organize chromosomes within the nucleus or impact chromosome pairing in somatic cells. Cytological analyses of interphase chromosome organization and pairing in these mutants are being used to address these hypotheses.

Interestingly, a previous screen for modifiers of *bw^p* PEV failed to produce any mutations that acted specifically on the *trans*-inactivation of the *bw⁺* allele seen with *bw^p* (TALBERT *et al.* 1994). However, the Talbert screen looked specifically for mutations that dominantly affect *bw^p* and did not affect the *cis*-inactivation of *bw*; this eliminated isolation of general modifiers of *bw* expression and general modifiers of PEV. No modifiers that behaved in such a fashion were identified. The two

mutations we have identified that specifically affect *bw^p* and TDA-PEV (*E69* and *E113*) are both X-linked recessive enhancers and would therefore have not been found in the Talbert screen. Since these *bw^p* modifiers are both recessive, it is possible that recessive autosomal mutations that affect *bw^p* could also be found. Further analyses of these mutations may provide insight into their roles in *bw^p* PEV specifically and in chromosome nuclear organization in general.

Two mutations, *26* and *E226*, alter only PEV associated with *yellow* expression [*i.e.*, TDA-PEV and *P(y⁺)*, Table 2]. These mutations are likely to identify genes associated with general *yellow* expression, rather than PEV. A more interesting behavior involves mutations *1309*, which affects TDA-PEV and only *w^{m4}*, and *E1451*, which impacts TDA-PEV and only *P(cen)*. Such differential behavior suggests that although *w^{m4}* and centric *P*-insertion silencing must have many components in common [general *Mod(var)s*], they also may have unique components. Alternatively, these mutations may identify proteins that function generally in PEV, but with lesions in specific residues or domains that play distinct roles in different types of silencing. Studies of such unique mutations as *1309* and *E1451* are likely to provide insight into the complex regulation of heterochromatin formation and function.

A new collection of general modifiers of PEV: In the last 20 years, multiple screens have been undertaken to identify mutations that modify position effect variegation (REUTER and WOLFF 1981; SINCLAIR *et al.* 1983; LOCKE *et al.* 1988; WUSTMANN *et al.* 1989; DORN *et al.* 1993). The majority of these screens focused on mutations that alter *w^{m4}* PEV. Most TDA-PEV modifier mutations also modify *w^{m4}*, suggesting that this collection of PEV modifiers may overlap significantly with those from previous screens. Some of these general *Mod(var)s* may be new alleles of previously identified modifiers of PEV, as described above for *1699/Su(var)3-9*. Indeed, one complementation group (*1009/1097/1207/1545*) is composed of new alleles of a previously identified modifier, *Su(var)2-5*. Analysis of other TDA-PEV modifiers may identify additional new alleles of previously identified modifiers of PEV.

However, the collection of general *Mod(var)s* described here is also likely to contain novel genes involved in gene silencing in *Drosophila*. Previous screens for general PEV modifiers did not saturate the genome, since many mutations appear to be solitary alleles of a specific locus (LOCKE *et al.* 1988; SINCLAIR *et al.* 1992; DORN *et al.* 1993). In addition, many of the TDA-PEV modifier mutations display subtle effects on *w^{m4}* and would likely have been missed in previous screens that focused on "strong" modifiers of *w^{m4}* PEV (REUTER and WOLFF 1981; SINCLAIR *et al.* 1983). Therefore, by using a different PEV type to identify *Mod(var)s* (*i.e.*, *y⁺* and TDA-PEV instead of *w^{m4}*), we have likely identified previously undetected modifiers of PEV.

One subset of these mutations is certainly unique: the 25 mutations that map to the *X* chromosome. Very few *X*-linked *Mod(var)*s have been identified in previous screens. In some cases, screens were designed to identify only autosomal mutations (SINCLAIR *et al.* 1983; LOCKE *et al.* 1988). In other cases, screens were designed such that dominant *X*-linked modifiers of PEV could be recovered (REUTER and WOLFF 1981; DORN *et al.* 1993; TALBERT *et al.* 1994), yet no such *trans*-modifiers were identified. Although our screen was specifically designed to allow the identification of recessive *X*-linked modifiers, only 8 of our 25 *X*-linked mutations are recessive. The remaining 17 are dominant mutations and therefore could presumably have been identified in previous screens; it is not clear why they were not. Regardless, the *X*-linked *Mod(var)*s described here provide a collection of new candidate silencing genes.

No specific modifiers of *trans*-suppression were recovered: Our screen was designed to identify mutations that altered TDA-PEV or *trans*-suppression, yet none of the 70 mutations specifically affect *trans*-suppression and not TDA-PEV. Why were we unable to obtain modifiers of *trans*-suppression? First, mutations affecting *trans*-suppression may reduce viability. Chromosome pairing appears to be important for gene function (ARAMAYO and METZENBERG 1996; LASALLE and LALANDE 1996; MORRIS *et al.* 1999), and mutating proteins involved in chromosome pairing may interfere with the expression of genes required for viability. Second, as mentioned above, we have not mutagenized to saturation, making it less likely we would have recovered mutations in rare genes. Third, our screen would uncover predominantly dominant autosomal modifiers, such that only mutations in genes that are dosage sensitive were identified; a screen for recessive mutations might identify loci specifically involved in *trans*-suppression. Finally, our definition of a modifier of *trans*-suppression is a mutation that causes decreased y^+ expression when $\gamma 878$, y^+ is in the presence of $\gamma 158$, γy^+ , but that has no effect on y^+ expression in animals that carry $\gamma 878$, y^+ alone. Proteins involved in *trans*-suppression may also be involved in TDA-PEV. Cytological analyses of modifiers of TDA-PEV may identify mutations that interfere with pairing of $\gamma 878$, y^+ and $\gamma 158$, γy^+ , thereby affecting *trans*-suppression.

In summary, we have isolated a novel group of *X*-linked and autosomal modifiers of PEV; this collection will be useful to the study of heterochromatin and telomere structure and function, nuclear organization, and the long-distance regulation of gene expression. Our secondary analyses of the mutations indicate that they are a diverse group, affecting a variety of PEV classes, and that these mutations may provide insight into the mechanisms of and overlap between different types of PEV. The mutations that specifically affect TDA-PEV or TDA-PEV and *bw^D* are of greatest interest for the study of telomere function and the organization of chromo-

somes in the nucleus. The roles of these proteins in nuclear biology will be determined by molecular cloning and investigation of the distribution and biochemical functions of the proteins, as well as by cytological analyses of the effects of homozygous mutations on nuclear and chromosome structure and function.

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