Mutations in the Protein Phosphatase 1 Gene at 87B Can Differentially Affect Suppression of Position-Effect Variegation and Mitosis in *Drosophila melanogaster*

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ABSTRACT

The suppressor of position effect variegation (PEV) locus *Su-var(3)6* maps to 87B5–10. The breakpoints of deficiencies that define this interval have been placed on a 250-kb molecular map of the region. The locus is allelic to the *ck19* complementation group previously shown to encode a type 1 serine-threonine protein phosphatase (PP1) catalytic subunit. When introduced into flies by *P* element-mediated transformation, a 5.8-kb genomic fragment carrying this gene overcomes the suppressor phenotype of *Su-var(3)6*^{01} and recessive lethality of all mutations of the locus. Four of the mutant alleles at the locus show a broad correlation between high levels of suppression of PEV, a high frequency of aberrant mitosis and low PP1 activity in larval extracts. However, some alleles with low PP1 activity show weak suppression of PEV with a high frequency of abnormal mitosis, whereas others show strong suppression of PEV with normal mitosis. The basis for these disparate phenotypes is discussed.

CHROMOSOMAL rearrangements that move euchromatic regions into the vicinity of constitutive heterochromatin may result in the euchromatic genes becoming inactive. Such rearrangements result in a variable degree of spreading of the heterochromatic nature of the chromosome from the breakpoint into the juxtaposed euchromatic region. At a certain stage of development, the degree of heterochromatinization becomes fixed and is then clonally inherited. The phenotypically visible variegation for a gene located in the affected euchromatic region is an indication of cell clones with or without heterochromatinization. The variable expression of a euchromatic gene transposed to a region of heterochromatin is referred to as position-effect variegation (PEV) (EISSENBERG 1989; SPRADLING and KARPEN 1990).

Dominant suppressor and enhancer mutations of PEV have been isolated in several laboratories (REUTER and WOLFF 1981; SINCLAIR, MOTTUS and GRIGLIATTI 1983; LOCKE, KOTARSKI and TARTOF 1988; WUSTMANN *et al.* 1989; DORN *et al.* 1993). Molecular studies have suggested three genes that modify PEV encode proteins that might be expected to affect chromatin structure. One of the two suppressor genes to have been cloned encodes a heterochromatin specific protein (EISSENBERG *et al.* 1990). Another suppressor of PEV affects H4 histone deacetylation (DORN *et al.* 1986), indicating that some modifier loci might code for gene products involved in the modification of chromosomal proteins.

The suppressor of PEV *Su-var(3)6* is a haplo-dependent suppressor of position-effect variegation (PEV) without a triplo enhancer effect that has been previously mapped to the 87B5–10 region on the right arm of the third chromosome (REUTER *et al.* 1987). It is allelic to the lethal complementation group *ck19* (GAUSZ *et al.* 1981). Recently, AXTON *et al.* (1990) demonstrated that the *e211* and *hs46* alleles of the *ck19* complementation group show abnormal mitosis in the brains of third instar larvae. The lethal phenotype of these mutants was rescued and normal mitosis restored by a 6.5-kb fragment containing the wild-type PP1–87B gene, but not when the gene carried a nonsense mutation. The PP1 87B gene encodes one of the four isoforms of the protein phosphatase I catalytic subunit (DOMBRADI *et al.* 1989). DOMBRADI *et al.* (1990a) showed that the alleles *e078*, *e211*, *hs46* have reduced PP1 activity, and that the *e211* mutation is a deletion that eliminates the promoter of the PP1 87B gene. However, mitosis is not disrupted in the hemizygous *e078* mutant, which has some residual phosphatase activity. Nevertheless, *e078*

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has a lethal phenotype that was rescued by the same genomic fragment encoding PP1 (AXTON *et al.* 1990).

In this paper we localize the *Su-var(3)6* gene cytologically with respect to chromosomal deficiencies, and at the molecular level with respect to a 250-kb chromosomal walk in the 87B8–10 region, which we have tied to the cytological markers. We show by *P* element-mediated transformation that the PP1 87B gene and *Su-var(3)6* co-localize on a 5.8-kb fragment. Finally, we present an analysis of the PP1 activity, suppression of PEV, and the mitotic phenotype in an allelic series of PP1 87B mutants.

MATERIALS AND METHODS

Stocks and mutations: Flies were raised at 25° on standard Drosophila medium. Description of the chromosomes and mutations used in this study can be found in LINDSLEY and ZIMM (1992) or in Table 1. Larvae with the correct phenotype were selected with the aid of *TM6B* balancer chromosome, which carries the dominant larval marker mutation *Tubby* (*Tb*).

Isolation of new deficiencies with breakpoints in the 87B region: Males of the genotype *In(3R)Na/Sb* were irradiated with 4.000 R of X rays and mated en masse to *cu kar/cu kar* females. The F_1 progeny (*In(3R)Na*/cu kar* or *Sb*/cu kar* genotypes) were scored for the presence of *kar* eye color. Males with *In(3R)Na/cu kar* genotype were crossed to *Df(3R)E-079/MKRS* females and their progeny scored for the presence of *Df(3R)E-079/In(3R)Na* category. In the absence of these flies, stocks were established over *MKRS* balancer chromosome. If the putative deficiency was induced on the *Sb*-bearing chromosome, the flies were crossed to *Df(3R)E-079/TM2* females; and if the *Sb/Df(3R)E-079* category was missing from the offspring, a stock was generated over the *TM2* balancer. If the F_1 *karmoisin* flies were females, their F_2 *karmoisin* male progeny were used for testing and stocks constructed as described above. The putative deficiency bearing stocks were crossed to representative lethal alleles from the *ck16, ck17, ck18, ck19* and *ck20* complementation groups (GAUSZ *et al.* 1981). About 20,000 F_1 flies were scored to recover two deficiencies (*Df(3R)SB3* and *Df(3R)SI55*) with breakpoints in the 87B region.

Isolation of X-ray-induced mutations: *In(3R)Na/Sb* male flies were irradiated with 4,000 R of X rays and mated en masse to w^{m4h}/w^{m4h} virgins. The males from F_1 progeny were screened for the presence of flies showing suppression of PEV. The putative suppressors were crossed to w^{m4h}/w^{m4h};*e168/TM3* females. PEV suppressor effect and lethality over the *ck19* allele (*e168*) was tested in male progeny. Stocks were established over the *TM3* balancer and tested with representative *ck16, ck17, ck18* and *ck20* alleles for lethality. Two of the isolated PEV suppressors (*1311* and *1455*) proved to carry lethal mutations in the *ck19* complementation group. Both the deficiency and mutant isolation was carried out on the *In(3R)Na* chromosome since we also wanted to map the chromosomal region responsible for the appearance of a large stage-specific puff in this stock.

Molecular studies: A genomic library was constructed from the *In(3R)Na/LS13* strain by partially digesting genomic DNA with *Sau*3A and ligating the fragments into the *Bam*HI cloning site of EMBL4 phage (FRISCHAUF *et al.* 1983). The chromosomal walk was conducted according to BENDER *et al.* (1983). The initial probe specific for the 87B5–10 region (BERNSTEIN *et al.* 1981) was kindly provided by S. I. BERNSTEIN. Genomic DNA and total RNA were prepared from adults and larvae as described by JOWETT (1986). Standard molecular techniques, including Southern and Northern analysis, were performed according to SAMBROOK, FRITSCH and MANIATIS (1989). Probes were generated by random oligonucleotide priming (FEINBERG and VOGELSTEIN 1983).

***P* element-mediated germ line transformation and mutant rescue:** A 5.8-kb *Eco*RI fragment carrying the *Su-var(3)6* wild-type gene was isolated from a recombinant phage (R3/18) and cloned into the unique *Sal*I site of Carnegie 20 transformation vector (RUBIN and SPRADLING 1983) by blunt end ligation. Transformation was performed according to RUBIN and SPRADLING (1982). Transformed ry^+ G1 flies were selected and balanced lines established using *CyO* and *TM3,*ry^{RK}*Sb e* balancer chromosomes.

The transformed lines were shown to contain the 5.8-kb insert by Southern hybridization. *In situ* hybridization with the biotinylated 7.3-kb *Hin*dIII fragment of the ry^+ gene was performed to locate the chromosomal insertion sites. The effect of the transforming DNA on the suppressor phenotype of *Su-var(3)*6^{01} was studied after crossing w^{m4h}; *Su-var(3)*6^{01}*/TM3* females with *+/Y; CyO/P[(*ry^+*)5.8 kb Eco R1]:*ry^{506} or *+/Y; +/+; P[(*ry^+*)5.8 kb Eco R1]/TM3,* ry^{RK}*Sb e* males, respectively. The amount of eye pigment in the *Su-var(3)*6^{01}-bearing offspring with and without the transformed fragment was measured. Rescue of recessive lethality was tested by crosses of *+/+, MKRS* or *TM3/lethal* or deficiency females with *P[(*ry^+*)5.8 kb EcoR1]/+; TM2/lethal* or deficiency males. Viability of transheterozygote *P[(*ry^+*)5.8 kb EcoR1]/+; lethal/deficiency or P[(*ry^+*)5.8 kb Eco R1]/+; lethal/lethal* flies indicates rescue of the recessive lethality of *Su-var(3)6* mutant alleles.

Germ-line clonal analysis: Germ-line mosaics were generated by pole cell transplantation (VAN DEUSEN 1976). Donor embryos derived from a cross of *Su-var(3)*6^{01} *red e/TM3* females to *Df(3R)E-079/TM3* males. Host embryos originated from crosses between wild-type females and *Fs(1)K1237/Y* males. The *Fs(1)1237* mutation causes a germ line dependent dominant female sterility (KOMITOPOLOU *et al.* 1983). Females eclosing from the host embryos cannot produce offspring unless they received donor pole cells that produce viable germ cells. All females were crossed to w^{m4h}/*Y; e/e* males, and from the phenotype of offspring the genotype of transplanted pole cells (*TM3/Su-var(3)*6^{01}*red e, TM3/Df(3R)E-079, TM3/TM3* or *Su-var(3)*6^{01}*red e/Df(3R)E-079*) can be determined. Females not laying eggs were dissected and their ovaries inspected for egg chambers developing further than that of *Fs(1)K1237* females, which stop at stage 4 (KING 1970).

Measurement of protein phosphatase 1 specific activity: Protein phosphatase activity and protein concentration of third larval instar and adult extracts was determined as described in DOMBRADI *et al.* (1990a). Phosphatase activity was measured using rabbit skeletal muscle phosphorylase A as substrate. This activity was sensitive to 100 U inhibitor protein-2 and so was attributed to protein phosphatase 1. This assay reflects the phosphatase activity of the highly conserved PP1 catalytic subunit. The physiological substrates in *Drosophila* cells are unknown and the regulatory proteins poorly characterized (see DISCUSSION).

Neuroblast squashes: *lethal/Df(3R)E-079* larvae from crosses between *Df(3R)E-079/TM6B* virgin females and *lethal/TM6B* males were grown at 25°. Larval ganglia were dissected in 0.7% NaCl and squashed in 45% acetic acid, dehydrated in ethanol and stained in 5% Giemsa's stain in 100 mM sodium phosphate, pH 7. Squashes were mounted

TABLE 1

Mutations and deficiencies in the region 87B and their effect on w^{m4h} PEV

Mutation	Source	Cytology	Origin	Effect on w^{m4h} R^a	PEV phenotype
Su-var(3)6^{01}	EMS	Normal	(1)	48.0	Su
Df(3R)E-079	EMS	86F1-2;87B8-10	(2)	9.2	Su
DF(3R)SB3	X rays	87B5-8:87C8-D1.2	(3)	23.3	Su
Df(3R)SI55	X rays	87B8-10;87D11-E1.2	(3)	2.3	Weak Su
1311	X rays	Normal	(3)	21.5	Su
1455	X rays	Normal	(3)	16.7	Su
e078	EMS	Normal	(2)	18.4	Su
e211	EMS	Normal	(2)	2.8	Weak Su
e168	EMS	Normal	(2)	4.7	Weak Su
hs16	EMS	Normal	(2)	2.8	Weak Su
hs46	EMS	Normal	(2)	2.9	Weak Su

The effect of the mutations in PEV was quantified by measuring red eye pigment in w^{m4h}/Y males after outcrossing homozygote w^{m4h}; +/+ females with +/*Y*; *Mutant/Balancer* males (REUTER AND WOLFF, 1981). R^a indicates the relative eye pigment content of mutant/+: +/+ w^{m4h} files. The subjective assessment of suppression is indicated as Su. The origins of the stocks are described in (1) REUTER *et al.* 1986; (2) GAUSZ *et al.* 1981; (3) this paper.

in Euparal and examined with a 63× objective without phase contrast.

RESULTS

Cytogenetic and molecular mapping of *Su-var(3)6*: We have localized the *Su-var(3)6* locus with respect to several chromosomal deficiencies, the breakpoints of which we have determined on a 250-kb chromosome walk. The *Su-var(3)6* locus maps to the overlapping region of the deficiencies *Df(3R)SB3* and *Df(3R)E-079* that uncover three lethal complementation groups (*ck17*, *ck18* and *ck19*) in the 87B5–10 bands (GAUSZ *et al.* 1981). Alleles of *ck17* and *ck18* do not show any suppressor effect on PEV. Mutations in *ck19* such as *1311*, *1455* and *e078* are strong suppressors (Table 1). Another deficiency, *Df(3R)SI55* extending distally fails to complement *ck19* alleles, thus placing the locus in 87B8–10. This deficiency displays only a very weak suppressor effect. The transheterozygotes of *Df(3R)SI55*, *1311* and *hs16* show a temperature sensitive partial complementation, whereas *e211* and *1455* give very few transheterozygotes (3/129 and 2/264, respectively) with the semilethal *Su-var(3)6^{01}* (Table 2). *Df(3R)SB3* must delete the *Su-var(3)6* locus completely since it does not complement any of the loci from *ck17* on the left of *Su-var(3)6* to the *kar* locus on the right. *Df(3R)E-079* and *Df(3R)SI55* may lack the gene entirely or partially because they overlap in *ck19* (Figure 2). The proximal breakpoint of *Df(3R)SB3* is to the left end of phage L1/10, marked as position 0 on the map of Figure 1. We have localized the proximal breakpoint of *Df(3R)SI55* and the distal breakpoint of *Df(3R)E-079* to the molecular intervals 59.4–59.6 and 62–64 kb, respectively (see legend to Figure 1). These results also locate the *Su-var(3)6* gene at around 60 on the DNA map.

P element transformation with the *Su-var(3)6* gene: Axton and co-workers have previously shown that the lethal (and, for some alleles, mitotic) phenotypes of *ck19* alleles could be rescued by germ-line transformation with a 6.5-kb fragment containing the protein phosphatase 1 catalytic subunit gene from 87B. Unfortunately, their transformation construct utilized the *white* gene as a marker, and so their transformants were unsuitable for testing whether this fragment would also rescue the *Su-var(3)6* phenotype in our assay. We therefore carried out transformation experiments using the 5.8-kb *Eco*RI (one of the *Eco*RI site derives from the EMBL4 vector) fragment from phage R3/18 (map position 56.2–62.0) inserted into the Carnegie 20 transformation vector, which uses *rosy* as transformation marker. This fragment also carries the PP1 87 gene. Three independent transformant lines carrying this 5.8-kb fragment were recovered. Southern analysis and *in situ* hybridization clearly showed the presence of independent insertion sites in transformants *P[(ry$^+$)5.8 kb] A, B and C* (Table 3). The effect of the transformed fragment on the suppressor phenotype of *Su-var(3)6^{01}* was studied by measuring eye pigment content in w^{m4h} background. All the transformants quench the strong suppressor effect of *Su-var(3)6^{01}* (Table 3). The same effect is found with tandem duplications covering this suppressor locus (REUTER *et al.* 1987). The rescue of the mutant phenotype indicates that the 5.8-kb fragment used includes both the essential coding and regulatory sequences of the *Su-var(3)6* gene. Flies with two additional copies of the *Su-var(3)6* locus show normal mottling, suggesting that the locus is without a triplo-dependent effect (Table 3).

Rescue of the recessive lethality associated with *Su-var(3)6* locus was tested by crossing balanced *MKRS/lethal* or *TM3/Df* females to +/*P[(ry$^+$)5.8 kb Eco*RI]; *TM2/lethal* or *P[(ry$^+$)5.8 kb Eco RI]/+; TM2/Df* flies.

TABLE 2

Complementation analysis of ck19 alleles and deficiencies uncovering the locus

	Df(3R)SB3	*Df(3R)SI55*	*Su6^{01}*	*1311*	*1455*	*e078*	*e168*	*e211*	*hs16*	*hs46*
Df(3R)E079	−	−	−	−[2]	−[2]	−	−	−	−	−
Df(3R)SB3		−	−	−[2]	−[2]	−	−	−	−	−
Df(3R)SI55			−[t1]	−[2]	−[2]	−	+	−	−	−
Su6^{01}				+	−[t2]	+	+	−[t3]	+	−
1311					−[2]	−	−	−	−	−
1455						−	−	−	−	−
e078							−	−	−	−
e168								−	−	−
e211									−	−
hs46										−[1]

In this table, + indicates a semilethal, and − a lethal combination. Superscripts have the following meanings.
[1] Both alleles were induced on the recessive lethal chromosome *Df(3R)kar^{3J}*.
[2] Alleles were induced on the *In(3R)Na* chromosome, which contains a second site lethal mutation.
[t1] This combination is lethal at 25° (0/80), and semilethal at 29° (7/50).
[t2] This combination gives no transheterozygotes at 25° (0/192), and very few at 29° (2/264).
[t3] This combination is lethal at 25° (0/136), and semilethal at 29° (8/129).

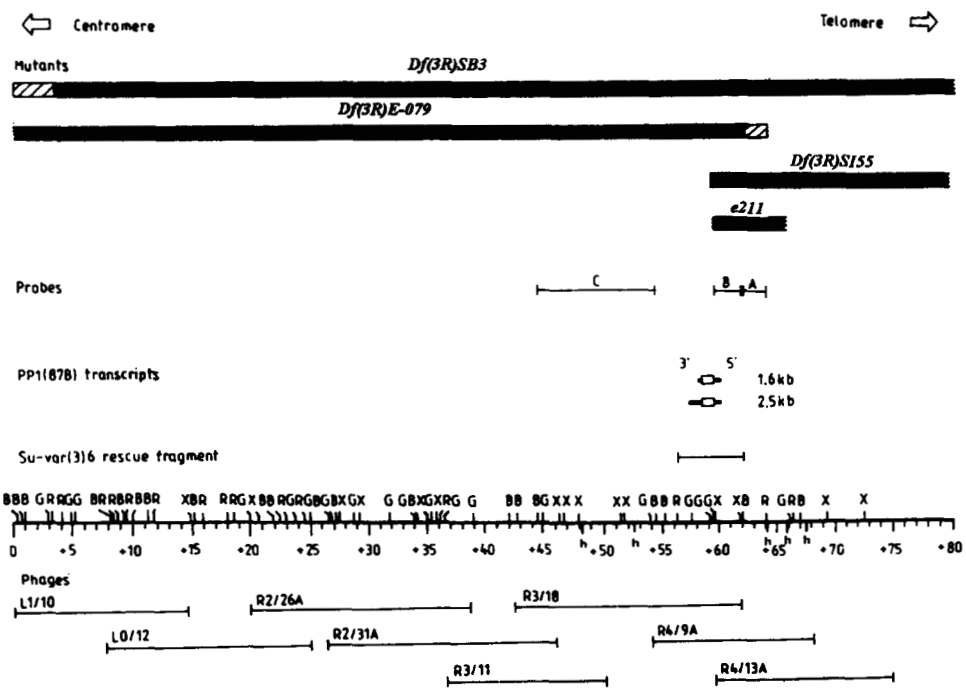

FIGURE 1.—Localization of *Su-var(3)6* gene on the DNA map. The chromosomal region 87B8–10 represented by recombinant phages. Missing fragment(s) distal from map position 0 in transheterozygotes of *Df(3R)SI55/Df(3R)E-079* were identified by comparing the restriction pattern of their DNA with wild-type genomic DNA. The 2.3-kb *Xho*I fragment from phage R3/18 (map position: 59.6–61.9) failed to hybridize with any fragment(s) from *Df(3R)SI55/Df(3R)E-079*. Consequently, the breakpoint of *SI55* is proximal, whereas the breakpoint of *E-079* is distal to this *Xho* I fragment. *Df(3R)SI55* ends in the 8.7-kb *Xho* I fragment (map position: 50.9–59.6). As we were able to detect the 0.3-kb *Bgl* II fragment (map position 59.3–59.6) in *Df(3R)SI55*, the breakpoint of this deficiency is located in the 59.3–59.6 genomic interval. The breakpoint of *Df(3R)E-079* lies in the 2-kb *Bam* HI-*Eco*RI fragment of phage R4/9A (map position: 62–64). In agreement with the results of AXTON and co-workers (1990), *e211* has also been shown to be deficient in the *E-079/SI55* overlap with a proximal break in the 2.3-kb *Xho*I fragment (map position 59.6–61.9). In the allelic mutants *1311*, *1455*, *e078*, *Su-var(3)6^{01}*, we were unable to detect any lesion in the relevant regions by Southern analysis. However, we might have failed to observe minor changes since analysis of the genomic interval between map positions 55.5–57.5 was hindered by the repetitive nature of this genomic region. Coordinates are given in kb on the DNA map. G: *Bgl* II; B: *Bam* HI; R: *Eco* RI; X: *Xho* I; H: *Hin*d III (*Hin*d III sites are shown only for the region corresponding to R3/18 and R4/9A phage clones). Deficiencies are indicated by filled bars, uncertainty of breakpoints is marked by hatched bars. Probes A, B and C are DNA fragments used for Southern and Northern hybridization. The 5.8-kb *Eco*RI fragment was used to resuce the suppressor phenotype of *Su-var(3)6^{01}* and the lethal phenotype of *ck19* alleles. PP1 (87B) transcripts are shown for comparison (based on DOMBRADI *et al.* 1990a).

Viability of *P[(ry^{+})5.8 kb Eco*RI]/+; *lethal*/deficiency; or *P[(ry^{+})5.8 kb Eco RI]; lethal/lethal* indicates rescue of the lethal phenotype. Lethal rescue was found in transheterozygotes of *Df(3R)E-079* with *Su-var(3)6^{01}*, *e078*, *e168*, *e211*, *hs16*, *hs46*, *1311*, *1455* and also with *Df(3R)SI55*. The same was found in transheterozygotes of the different mutations. The lethality of *Df(3R)E-079/Df(3R)SI55* was also rescued by the 5.8-

TABLE 3

Rescue of the suppressor phenotype of *Su-var(3)6^{01}* and the effect of additional wild-type copies of the gene on PEV in *w^{m4h}*

Transformant line or duplication	Cytology	Genotype[a]	Relative eye pigment content	Number of *Su$^+$* loci	*R*[b]
P[(ry$^+$)5.8 kb]A	52E1.2–5.6	*5.8 kb A/+;Su/+*	26.5 ± 4.5[c]	2	2.7
		CyO/+;Su/+	72.2 ± 6.5	1	
P[(ry$^+$)5.8 kb]B	78E	*5.8 kb B/Su*	23.6 ± 5.3[c]	2	3.5
		Su/TM3	83.6 ± 6.2	1	
P[(ry$^+$)5.8 kb]C	61D	*5.8 kb C/Su*	30.2 ± 4.8[c]	2	3.0
		Su/TM3	89.1 ± 7.6	1	
Dp(3;3)D1	86E1;87C3–5	*Su/Dp*	26.4 ± 5.8[c]	2	2.5
		Su/TM3	67.1 ± 2.1	1	
P[(ry$^+$)5.8 kb]A	52E	*Sco/+TM3/+*	2.6 ± 0.3	2	
+	+	*Sco/+;5.8 kb B/+*	2.4 ± 0.4	3	0.9[e]
P[(ry$^+$)5.8 kb]B[d]	78E	*5.8 kb A/+;5.8 kb*	2.5 ± 0.3	4	1.0[e]

[a] Females of the genotype *w^{m4h};Su-var(3)6^{01}/TM3* were crossed to *+/Y;P[(ry$^+$)*5.8 kb EcoR1]A/CyOL;*ry^{506}* or +/Y;+/+;*P[(ry$^+$)5.8 kb EcoRI] B* and *C/TM3l;ry^{RK}Sb e* males.

[b] Ratio of the relative eye pigment content of *Su* males lacking the insertion *P[(ry$^+$)5.8 kb EcoRI]* to those carrying it.

[c] The *Su-var(3)6^{01}* mutant shows a significant maternal effect; when *Su-var(3)6^{01}* is inherited from the male parent the amount of the red eye pigment measured is about 10 times less.

[d] *w^{m4h};P[(ry$^+$)5.8 kb EcoRI]A/Sco;+/+* females were crossed to *+/y;P[(ry$^+$)5.8 kb EcoRI]B/TM3* males.

[e] Ratio of relative pigment content of *w^{m4h}/Y* males with one and two additional *Su$^+$* copies compared with *w^{m4h}/Y;+/+* flies.

kb fragment, indicating that there are no lethal mutable loci in the overlap other than the one contained in the rescue fragment (*i.e.*, *ck19*). (In the chromosomes *e078*, *e168* and *e211* independent second site lethals have to be present because heterozygotes with the deficiencies were rescued whereas homozygotes remained lethal.)

Female germ-line function of *Su-var(3)6^{01}*: Previous complementation studies indicated that the viability of *Su-var(3)6^{01}/e078* transheterozygotes is dependent on the maternal inheritance of *Su-var(3)6^{01}* (REUTER *et al.* 1987). Maternal effect was also found in reciprocal crosses giving elevated pigment content in both *Su* and *Su$^+$* offspring if the suppressor mutation is maternally inherited (Table 3). We therefore decided to test the lethality of *Su-var(3)6^{01}/Df* transheterozygotes during female germ-line development by pole cell transplantation into the otherwise female sterile host *Fs(1)K1237* (see MATERIALS AND METHODS). Among the 69 females eclosed 32 were chimeras. Eleven females gave offspring from *Su/TM3*, nine from *Df/TM3* pole cells. In 12 females the genotype of the transplanted germ-line cells was *TM3/TM3*. Although several (8) hosts were expected to receive *Su-var(3)6^{01} red e/Df* pole cells, none were observed. After dissection of the sterile females, we did not find egg chambers developing further than stage 4 typical for *Fs(1)K1237* (KOMITOPOULOU *et al.* 1983). This is evidence that the *Su-var(3)6* gene product is essential for germ-line development.

Northern analysis: When we probed the RNA blots with the nonrepetitive 2.3-kb *Xho*I fragment from within the transforming fragment, two main RNA species of 2.5 kb and 1.7 kb were visualized (Figure 2). These two transcripts correspond to those of the

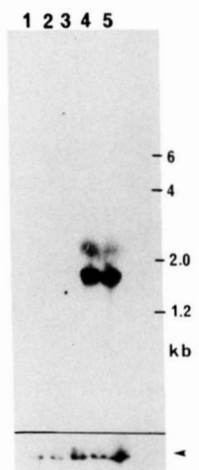

FIGURE 2.—Northern analysis of *Su-var(3)6* mutants. Total RNA samples prepared from third instar larvae were run on a 1% formaldehyde-agarose gel, blotted onto nitrocellulose, and hybridized with the 2.3-kb *Xho*I genomic clone (Figure 1, probe B). The arrowhead indicates rehybridzation of the same filter with a 5C specific actin clone. Lanes: 1, *1311/E-079*; 2, *1455/E-079*; 3, *SI55/E-079*; 4, *Su-var(3)6^{01}/Su-var(3)6^{01}*; 5, *w^{m4h}/w^{m4h}*.

PP1 87B gene (AXTON *et al.* 1990). These two transcripts were missing from *Df(3R)E079/Df(3R)SI55* larvae, and from hemizygotes of *1311* and *1455* (Figure 2), but they were present in *Su-var(3)6^{01}* homozygotes and in hemizygotes of *Su-var(3)6^{01}*. AXTON *et al.* (1990) previously showed that these two transcripts are present in larvae hemizygous for *e078* but are missing from larvae hemizygous for *e211* and are reduced in size in *hs46* larvae.

Protein phosphatase 1 activity of *Su-var(3)6* mutants: In all *ck19* alleles tested, the PP1 specific activity was reduced compared with the respective wild-type levels in larvae (Table 4). The PP1 activity of homozygous and transheterozygous *Su-var(3)6^{01}* larvae and

TABLE 4

Protein phosphatase 1 activity in *Su-var(3)6* alleles

Allele/strain	Stage	Specific activity mU/mg ± SD	(*n*)
Canton S	A[a]	6.60 ± 0.70	(10)
w^{m4h}/w^{m4h}	A	5.39 ± 0.86	(10)
w^{m4h};*Su-var(3)6*^{01}/*TM3*	A	3.89 ± 0.41	(8)
w^{m4h};*Su-var(3)6*^{01}/*Su-var(3)6*^{01}	A	2.39 ± 0.52	(7)
Canton S	L[b]	9.53 ± 0.61	(7)
w^{m4h}/w^{m4h}	L	7.25 ± 0.58	(5)
w^{m4h};*Su-var(3)6*^{01}/*Su-var(3)6*^{01}	L	1.52 ± 0.40	(7)
Su-var(3)6^{01}/*Df(3R)E-079*	L	1.18 ± 0.27	(9)
Su-var(3)6^{01}/*Df(3R)SI55*	L	2.14 ± 0.34	(8)
Su-var(3)6^{01}/*TM6B*	L	3.45 ± 1.55	(15)
In(3R)Na/TM6B	L	7.41 ± 0.42	(4)
In(3R)Na/Df(3R)E-079	L	6.41 ± 0.80	(6)
1311/Df(3R)E-079	L	1.17 ± 0.21	(7)
1311/TM6B	L	3.77 ± 1.03	(13)
1455/Df(3R)E-079	L	1.26 ± 0.36	(11)
1455/TM6B	L	4.03 ± 1.30	(15)

[a] A, Adults, [b] L, third instar larvae.

adults is nearly as low as that of the amorphic *ck19* mutants *1311*, *1455* (Table 4), *e211* and *hs46* (DOMBRADI *et al.* 1990a), indicating that mutations in all of these alleles affect the PP1 (87B) gene. The reduction of PP1 activity in *168* and *hs16* was less significant. The results obtained with *1311* and *1455* also confirm that PP1 (87B) is the major contributor to PP1 activity in third instar larvae and adults (cf. DOMBRADI *et al.* 1990a) (Table 4). The assays of adult *Su-var(3)6*^{01} mutants demonstrate that the 87B isoenzyme also represents the main PP1 activity in the adult stage (Table 4).

Abnormal mitosis shown by alleles of the *ck19* complementation group: Abnormal mitosis in larval neuroblasts has been previously reported for the *e211* and *hs46* alleles but not for *e078* (AXTON *et al.* 1990). We have therefore examined other mutant alleles of the locus for mitotic defects (Table 5). All ganglia from the hemizygous mutant larvae exhibited some neuroblasts or ganglion mother cells with the same mitotic defects seen in mutants bearing the null PP1 87B allele *e211* (AXTON *et al.* 1990). The aberrant figures most frequently showed overcondensed metaphase chromosomes, and separate, but overcondensed, anaphase chromatids. Hyperploid cells with overcondensed chromosomes were seen at a lower frequency.

It is possible to recognize three classes of mutant PP1 87B alleles based on the relative frequency of abnormal mitosis in larval neuroblasts (Table 5 and AXTON *et al.* 1990). The *e211*, *1311*, *1455*, *SI55* and *SB3* alleles were associated with the highest frequency (>40%) of aberrant metaphase and anaphase figures. *e168*, *hs16* have a weaker effect, with 10–30% aberrant figures. *Su-var(3)6*^{01} and *e078* had the weakest effect of all, resulting in less than 5% aberrant mitotic figures.

DISCUSSION

The *Su-var(3)6* locus has been mapped cytogenetically to 87B8–10 and several allelic mutations have been identified. These analyses together with the data of REUTER *et al.* (1987) have yielded a total of 11 mutations that affect the *Su-var(3)6* locus. Six of these (*Su-var(3)6*^{01}, *e078*, *1311*, *1455*, *Df(3R)SB3* and *Df(3R)E-079*) show clear suppression of PEV, and five (*e211*, *hs16*, *hs46*, *e168* and *Df(3R)SI55*) have a weak suppressor effect.

In this work we show that the cytologically visible deficiencies *Df(3R)E-079*, *Df(3R)SB3* and *Df(3R)SI55* are missing all or part of the PP1 87B gene. Of the *ck19* alleles, *e211* was previously shown by AXTON *et al.* (1990) to be an extensive deletion removing 5′ PP1 gene sequences, and *hs46* a smaller deletion. This was confirmed by DOMBRADI *et al.* (1990a), who showed that the *e211* deletion was greater than 6 kb, and by DOMBRADI and COHEN (1992), who determined that 474 bp of 5′ coding and noncoding sequences were missing from the *hs46* allele. This explains the lack of PP1 transcripts from the *e211* mutation and the shorter transcripts from *hs46* found by AXTON *et al.* (1990). From an analysis of the *e211* null mutant, DOMBRADI *et al.* (1990a) estimated that

TABLE 5

Abnormal mitotic figures in *Su-var(3)6* alleles

Genotype	Normal metaphase	Normal anaphase	Over-condensed metaphase	Over-condensed anaphase	Hyperploid	Over-condensed hyperploid	Total mitoses	Total ganglia	% aberrent mitoses
1455/Df(3R)E079	129	42	169	137	5	55	537	3	68.2
1311/Df(3R)E079	60	11	126	48	0	15	260	7	72.7
Df(3R)SI55/Df(3R)E079	27	11	41	10	0	1	90	5	57.8
Df(3R)SB3/Df(3R)E079	30	1	21	4	0	10	76	5	46.1
e168/Df(3R)E079	419	114	172	34	3	9	751	4	29.0
hs16/Df(3R)E079	401	129	45	23	0	2	600	4	11.7
Su-var(3)6^{01}/*Df(3R)E079*	478	199	16	8	1	0	702	5	3.5
Su-var(3)6^{01}	313	147	46	8	2	5	521	7	11.9

expression of the PP1 gene at 87B contributed about 80% of total PP1 activity in third instar larvae. The residual activity must result from isozymes encoded by genes at three other locations: 9C, 13C, and 96A (DOMBRADI *et al.* 1989a, b). A measurement of total PP1 activity does, however, give an indication of the effects of the mutations we are studying. The absence of transcripts and the basal level of PP1 activity in the *1311* and *1455* alleles suggests that these are also null alleles of the PP1 87B gene. The other two alleles that have been analyzed at the molecular level are *Su-var(3)6^{01}* and *e078*, which each have point mutations that change a conserved glycine residue to serine and aspartic acid, respectively (DOMBRADI and COHEN 1992; DOMBRADI, GAUSY and COHEN 1991). The molecular lesion of the *1311*, *1455*, *e168* and *hs16* alleles has yet to be determined, although our failure to observe transcripts from *1311* and *1455* indicates that the promoter is affected in these mutants. The effect of the mutations upon viability correlates with PP1 activity (DOMBRADI *et al.* 1990a, Table 4).

It was previously shown that the lethality of mutants *e211*, *hs46*, and *e078* could be rescued by a 6.5-kb genomic fragment containing the PP1 (87B) gene, but not by the equivalent fragment in which a nonsense mutation was introduced into the PP1 gene (AXTON *et al.* 1990). A slightly shorter fragment of 5.8 kb has been used in the present study. It also contains the PP1 (87B) gene and rescues the other lethal *ck19* mutants. The rescue of the lethal phenotype of transheterozygotes *Df(3R)E-079* and *Df(3R)SI55* with *Su-var(3)6* alleles proves that the lethality is the consequence of a mutation in the PP1 (87B) gene that results in reduced PP1 activity. The suppressor phenotype of the *Su-var(3)6^{01}* allele (a strong suppressor of PEV) is rescued by the same 5.8 kb genomic fragment. Therefore the 5.8 kb transforming fragment contains all the essential sequences for rescue of both the lethality and the suppression of PEV.

The importance of the maternally provided gene product in germ-line development was demonstrated by pole cell transplantation. This is consistent with the previous finding of REUTER *et al.* (1987) that *Su-var(3)6$^{ts;01}$* exhibits a maternal effect. A maternal effect parallel to the one found for PP1 was also demonstrated in reciprocal crosses giving elevated pigment content if the suppressor mutation was maternally inherited (Table 3).

For four of the PP1 87B mutant alleles, it is possible to correlate the effects of the mutation on mitosis of neuroblasts, PEV and phosphatase activity in whole larval extracts. *1311* and *1455* both show a high frequency of aberrant mitoses, strong suppression of variegation and low phosphatase activity in extracts. In these respects, these alleles resemble the deficiency for the entire locus *Df(3R)SB3* and may be considered null alleles. In contrast, *e168* and *hs16* have a moderate effect on mitosis, show weak variegation suppression and have slightly less than wild-type levels of activity.

However, a simple model in which different alleles affect only the quantity of PP1 activity does not account for several of our observations. DOMBRADI *et al.* (1990a) conjectured that residual activity found in *e078/Df(3R)E-079* larvae represented a threshold level needed for normal passage through mitosis. However, we find a very low frequency of abnormal mitosis in neuroblasts of *Su-var(3)6^{01}/Df(3R)E-079* larvae, along with phosphatase activity levels comparable to the null *1311* and *1455/Df(3R)E-079*. Alternatively, AXTON *et al.* (1990) proposed that the mitotic defects might result only when the catalytic subunit was totally absent, due to excess free regulatory subunits, perhaps interfering with the functioning of another PP1 isoenzyme or with other proteins essential for mitosis. This last model cannot provide the complete explanation because of the good correlation of loss of activity with frequency of abnormal mitosis in the case of the *e168* and *hs16* alleles, although these mutations could themselves prevent PP1 from fully interacting with regulatory subunits.

Consequently, at least two explanations are possible for the lack of effect of the *Su-var(3)6^{01}* and *e078* mutations on mitosis. On the one hand, the mutant protein encoded by these two alleles could still bind correctly to regulatory subunits, thus permitting normal mitosis. Biological activity associated with fragments of PP1 is not without precedent. The gene encoding one of two isoforms of PP1 in fission yeast (*dis2*) has been shown to be able to cause a *cdc25^{ts} wee1^{-}* double mutant to revert to a temperature-sensitive cdc phenotype (BOOHER and BEACH 1989). This activity has been mapped to a small C-terminal peptide region of the *dis2* PP1 (KINOSHITA *et al.* 1991) The mitotic role of this fragment of PP1 in fission yeast remains to be fully characterized. An alternative explanation of the mitotic phenotypes of the mutations *e078* and *Su-var(3)6^{01}* is that they affect substrate specificity of the enzyme. In this case, mitotic substrates would be dephosphorylated relatively normally, whereas dephosphorylation of phosphorylase (the substrate used for our *in vitro* assays) and the substrates having a role in PEV is reduced. It is presently unclear which regions of the catalytic subunit are responsible for the binding of interacting subunits thought to determine substrate specificity. Interacting subunits remain to be characterized in Drosophila, although in fission yeast the gene for one such protein (*sds22*) has been identified as a multicopy suppressor of the semi-dominant cold sensitive *dis2–11* mutation (OHKURA and YANAGIDA 1991). To separate the effects of mutations in the Drosophila genes

upon intrinsic activity of the catalytic subunit and on its ability to interact with its regulators, it will be necessary to assay the activity of the cloned mutant PP1 87B genes *in vitro*.

Our most intriguing finding is that of a class of mutant PP1 87B alleles that display a high frequency of abnormal mitosis when hemizygous but only a weak dominant PEV suppressor effect. All three of these mutations, *e211*, *hs46* and *Df(3R)SI55*, delete all or part of the 5′ end of the PP1 87B transcription unit while leaving most of the coding region intact. All were predicted to be null mutations: *e211/Df(3R)E-079* and *Df(3R)SI55/Df(3R)E-079* larvae lack PP1 87B RNA; and in *hs46/Df(3R)E-079*, transcripts are truncated and the predicted translational start site is missing. These observations are sufficient to explain the strong mitotic phenotype of these mutations and null activity in larval extracts. However, the weak *Su-var* phenotype of these alleles was unexpected. One possibility is that each of these three stocks bears an independent enhancer of PEV (*E-var*) on the same chromosome as the PP1 87B mutation. This seems unlikely since the genetic background of these three stocks is different. Furthermore, when segments of the chromosome carrying *e211* are exchanged through recombination, the suppressor phenotype is not significantly changed. However, preliminary results indicate that *Df(3R)SI55* does uncover a homozygous viable enhancer locus distal to *Su-var(3)6* that displays a significant paternal effect (DORN *et al.* 1993). Another possibility, despite the lack of PP1 87B RNA in mutant third instar larvae, is that sufficient truncated PP1 protein is produced from the mutated gene, perhaps from a cryptic promotor, at sufficient levels at a critical period of development to restore wild-type PEV. We are currently investigating both of these possibilities.

It is known that PP1 has broad substrate specificity and affects many vital functions probably through interaction with different substrate proteins and/or different targeting or regulatory proteins (COHEN 1989). Importantly, none of the other PP1 isoenzymes can substitute for PP1 (87B) in its role in mitosis or PEV, indicating a specialized function for the different PP1 isoforms in Drosophila. The broad substrate specificity of PP1 makes it difficult to predict which proteins it might regulate in PEV. One possible candidate is the zinc finger containing protein product of the *Su-var(3)7* locus that has about 40 potential phosphorylation sites (REUTER *et al.* 1990). Phosphorylation of the protein may prevent effective DNA binding and consequently heterochromatinization. Dephosphorylation by PP1 on the other hand may allow DNA binding and chromosome condensation. This is supported by the finding that the *Su-var(3)6*^{01} mutation is epistatic over the enhancer effect of additional wild-type copies of the *Su-var(3)7* locus (REUTER *et al.* 1990). Contrary to the previously cloned haplo-suppressors with triplo-enhancer effect, *Su-var(3)7* and *Su-var(2)5* (EISSENBERG *et al.* 1990), which code for structural components of the heterochromatin, *Su-var(3)6* without any triplo-enhancer effect codes for an enzyme, the properties of which suggest that the rate of phosphorylation-dephosphorylation of chromosomal proteins is in a delicate balance at the time at which heterochromatinization is set.

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