A Cytogenetic and Genetic Characterization of a Group of Closely Linked Second Chromosome Mutations That Suppress Position-Effect Variegation in *Drosophila melanogaster*

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ABSTRACT

Characterization of a group of dominant second chromosome suppressor of position-effect variegation (PEV) (*Su(var)*) mutants has revealed a variety of interesting properties, including: maternal-effect suppression of PEV, homozygous lethality or semilethality and male-specific hemizygous lethality, female infecundity, acute sensitivity to the amount of heterochromatin in the cell and sensitivity to sodium butyrate. Deficiency/duplication mapping and complementation tests have revealed that eight of the mutants define at least two genes in section 31 of the left arm of chromosome 2 and they suggest that a ninth corresponds to an additional nonessential *Su(var)* gene within or near this region. The effects of specific deficiencies and a duplication on PEV indicate that the expression of one or more of the *Su(var)* genes in this region of the chromosome is dose-dependent, *i.e.*, capable of haplo-abnormal suppression and triplo-abnormal enhancement. Interestingly, the appearance of certain visible phenotypes among a subset of the mutants suggests that they may possess antimorphic properties. Our results are consistent with the hypothesis that two of these *Su(var)* genes encode structural components of heterochromatin. We also report that two previously isolated mutants located in 31E and 31F-32A act as recessive suppressors of PEV.

IN its most common form, position-effect variegation (PEV) is a type of somatic mosaicism associated with inactivation of a gene that has been relocated close to a heterochromatic breakpoint (reviewed by SPOFFORD 1976). Such relocated euchromatic regions often acquire a heterochromatic morphology and the extent of this heterochromatinization is correlated with the degree to which genes in these regions are inactivated (PROKOFYEVA-BELGOVSKAYA 1947; SCHULTZ 1956; HARTMANN-GOLDSTEIN 1967; KORNHER and KAUFFMAN 1986). The most plausible explanation for the mosaic phenotype is that in some cells of a variegated tissue, genes in heterochromatinized regions of the chromosome are transcriptionally inactive, whereas in other cells, these genes are packaged as euchromatin and expressed normally.

It has been suggested that the formation of heterochromatin occurs via a self-assembly process requiring the participation of histones, non-histone chromosomal proteins (NHPs) and DNA (SPOFFORD 1976). Presumably, under normal circumstances, the regional integrity of the chromosome is maintained by specific attributes of the *bona fide* heterochromatic/euchromatic junction and assembly of heterochromatin is restricted to the centromeric region. However, in the case of a variegating rearrangement, heterochromatic elements could spread illicitly across the newly formed breakpoint into adjacent euchromatic regions (ZUCKERKANDL 1974). If this hypothesis is correct, then the genetic and molecular study of PEV should provide insight into mechanisms that control chromatin and chromosome assembly, as well as the influence of chromatin structure on gene expression.

That the process of heterochromatin assembly is highly sensitive to the availability of structural components is inferred from the finding that heterozygous deletions of the histone genes suppress PEV (KHESIN and LEIBOVICH 1978; MOORE *et al.* 1979; MOORE, SINCLAIR and GRIGLIATTI 1983). The dramatic effects of addition or removal of heterochromatic elements, such as the *Y* chromosome, on the expression of variegating genes, may be another manifestation of this sensitivity (GOWEN and GAY 1934; and see ZUCKERKANDL 1974). A reasonable extension of this hypothesis is that mutations in genes encoding heterochromatic-specific NHPs will also suppress PEV.

More than 100 dominant autosomal mutations that suppress PEV in *Drosophila melanogaster* have been isolated (REUTER and WOLFF 1981; SINCLAIR, MOTTUS and GRIGLIATTI 1983; REUTER *et al.* 1986, 1987; WUSTMANN *et al.* 1989). Reuter and co-workers argue

We dedicate this paper to the memory of LARRY SANDLER.

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that there are at least 150 genes capable of influencing PEV (WUSTMANN *et al.* 1989). Cytological and biochemical evidence suggests that some of the *Su(var)* mutations can alter chromatin structure (REUTER, WERNER and HOFFMANN 1982; DORN *et al.* 1986; HAYASHI *et al.* 1990). It has been reported that one of the mutants from our collection, *Su(var)205*, affects the expression of a chromatin protein gene (JAMES and ELGIN 1986; EISSENBERG *et al.* 1990). In addition, REUTER *et al.* (1990) have determined that a third chromosome *Su(var)* gene encodes a zinc finger protein. These results are consistent with the hypothesis that some and perhaps many of the suppressor genes code for NHPs. Hence, genetic and molecular analyses of other *Su(var)* mutants and their genes should prove worthwhile.

Previous mapping experiments in our laboratory localized a group of dominant *Su(var)* mutants to a relatively discrete segment in the left arm (2L) of chromosome 2 (SINCLAIR, MOTTUS and GRIGLIATTI 1983). In this paper, we describe a comprehensive cytogenetic and genetic analysis of this group. Our data indicate that the mutants define three genes, two of which map within section 31 of 2L. In general, the mutants exhibit a common syndrome of phenotypes, the most striking of which include: dominant maternal effects, recessive lethality or semilethality, female infecundity, sensitivity to the amount of cellular heterochromatin, and butyrate sensitivity. The properties of the *Su(var)* loci are discussed in relation to the hypothesis that they encode NHPs.

MATERIALS AND METHODS

Mutant strains and chromosomes: Most of the mutant strains and chromosomes used in this study are described below. Additional information about many of these and other relevant mutants can be found in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1985, 1986, 1987).

First, two series of second chromosome rearrangements generated through reversion of the neomorphic mutation, *Jammed* (*J*) (see Figure 3); (a) *Df(2L)J2*, *Df(2L)J27* and *Df(2L)J39* (hereafter referred to as *Df2*, *Df27* and *Df39*, respectively), were kindly supplied by L. SANDLER (see MANGE and SANDLER 1973; SANDLER 1977). (b) *Df(2L)J233*, *Df(2L)J77*, *Df(2L)J106* and *T(Y;2)J99* (hereafter referred to as *Df233*, *Df77*, *Df106* and *T99*, respectively), were provided through the generosity of J. LENGYEL (see SALAS and LENGYEL 1984).

Second, *Dp(2;2)Mdh3, Sp (Dp(2;2)30D1-E1;32D1-F3)* (E. GRELL, personal communication) was kindly supplied by E. GRELL.

Third, a series of recessive lethal and/or female sterile mutations that map within the limits of *Df39*, was obtained from L. SANDLER: *daughterless* (*da*), *abnormal-oocyte* (*abo*), *daughterless-abnormal-oocyte-like* (*dal*), *wavoid-like* (*wdl*), *hold-up* (*hup*), *male-female-sterile-48* (*mfs48*) and *lethal(2)54* (*l(2)54*) [see MANGE and SANDLER (1973) and SANDLER (1977)].

Fourth, a group of dominant suppressor-of-variegation (*Su(var)*) mutants were induced in our laboratory, using the chemical mutagen ethylmethane sulfonate (EMS) (SINCLAIR, MOTTUS and GRIGLIATTI 1983): *Su(var)204*, *Su(var)207*, *Su(var)209*, *Su(var)210*, *Su(var)213*, *Su(var)214*, *Su(var)215* and *Su(var)216*. Hereafter, these are referred to as *204*, *207*, *209*, *210*, *213*, *214*, *215* and *216*, respectively. All of these mutations have been localized between map positions 32–35 on the left arm of chromosome 2.

Fifth, the *Suvar(2)1*^{01} mutation (referred to as *1*^{01}), is a spontaneous lesion that maps to position 40.5 in 2L (REUTER, DORN and HOFFMANN 1982). This strain was provided through the generosity of G. REUTER.

Culture conditions: Standard cornmeal-sucrose Drosophila medium, with tegosept added as mold inhibitor, was used. Except where indicated, cultures were maintained and crosses performed at 22°.

Phenotypic characterization of *Su(var)* mutants: Two types of variegation assays were used (for more specific details, see SINCLAIR, MOTTUS and GRIGLIATTI 1983). The first was a fluorometric measurement of levels of red pigment in the eyes of adults of various genotypes bearing *In(1)w*^{m4} or *In(2R)bw*^{VDe2} (hereafter referred to as *w*^{m4} or *bw*^{V}, respectively). The values obtained were expressed as percentages of pigment levels of control flies (usually Oregon R). The second was a visual determination of the extent of *Sb* variegation in adults bearing *T(2;3)Sb*^{V} (hereafter referred to as *Sb*^{V}). In this assay, the values were expressed as the percentage of *Sb* bristles among 14 major bristles examined per individual. The values are shown as mean percentage of full wild-type (*w*$^{+}$ or *bw*$^{+}$) or mutant (*Sb*) gene expression ± standard error. The sample size for both assays was 25.

In most cases, progeny assayed were obtained by crossing *w*^{m4}/*Y*; *Su(var)*-bearing males to females bearing one of the three variegating rearrangements. Specific exceptions to this mating protocol are described in RESULTS.

Cytology: Males bearing chromosome rearrangements within regions 31–32 were crossed to Oregon-R females and the offspring were raised at 17°. Salivary glands from third instar larvae were dissected in Drosophila saline, fixed in 45% acetic acid, stained in lacto-aceto-orcein (YOON, RICHARDSON and WHEELER 1973) and squashed. Chromosomes were examined under phase contrast optics and rearrangement breakpoints interpreted according to the revised map of BRIDGES (LEFEVRE 1976).

Deficiency mapping of *Su(var)* and other mutants: Deficiencies lacking segments in the 31–32 region of 2L (see Figure 3) were used to localize the *Su(var)* mutants, as well as several recessive mutants described by SANDLER (1977). Cultures from deficiency mapping crosses were examined for survival of *Df/Su(var)* offspring. In addition, the fecundity (ability to produce eggs) of surviving *Df/Su(var)* females was tested. This test was also used for crosses involving the female sterile deficiencies, since these cause maternal-effect lethality rather than lack of egg production (SANDLER 1977; D. SINCLAIR, unpublished observations). In several cases, recessive mutants were mapped in a conventional manner, *i.e.*, cytological localization was based on survival of the mutant (either males or both sexes) when heterozygous with particular deficiencies.

Tests for butyrate sensitivity: Specific *Su(var)* mutants were tested for sensitivity to sodium butyrate as follows. *w*^{m4}/*Y*; *Su(var)*/*CyO* males were crossed to *Df2/SM1, Cy* females (50–100 parents per cross) and synchronously developing embryos were collected on Petri plates containing normal medium or medium supplemented with 150 mM sodium butyrate. The embryos were counted and allowed to hatch. Then, the first instar larvae were transferred to fresh experimental or control medium and allowed to develop to eclosion.

TABLE 1

Phenotypic properties of *Su(var)* mutants from 2L group

Mutant	Map position	Homozygous viability[a]		Percent full gene expression w^{m4}	bw^V	Sb^V
204	33.8	Viable	f[b]	48 ± 2	36 ± 2	81 ± 2
			m	63 ± 4	33 ± 2	81 ± 3
207	32.0	Lethal	f	112 ± 6	95 ± 1	93 ± 1
			m	73 ± 5	94 ± 2	96 ± 1
209	35.4	Viable	f	77 ± 3	57 ± 6	62 ± 4
			m	70 ± 5	56 ± 8	77 ± 3
210	34.8	Lethal	f	82 ± 8	65 ± 2	71 ± 3
			m	82 ± 5	59 ± 3	79 ± 2
213	32.9	Lethal	f	89 ± 1	103 ± 10	83 ± 3
			m	89 ± 2	131 ± 7	89 ± 2
214	34.9	Semilethal	f	77 ± 4	107 ± 6	88 ± 3
			m	103 ± 4	106 ± 7	91 ± 4
215	32.9	Semilethal	f	79 ± 5	105 ± 6	80 ± 2
			m	73 ± 7	111 ± 6	90 ± 2
216	34.2	Lethal	f	101 ± 1	56 ± 4	
			m	38 ± 5	34 ± 1	
1^{01}[c]	40.5[c]	Semilethal	f	83 ± 1	11 ± 1	79 ± 3
			m	78 ± 1	13 ± 1	75 ± 3
Controls			f	5 ± 1	21 ± 1	54 ± 1
			m	10 ± 2	25 ± 1	55 ± 1

[a] See Figure 1.
[b] f = female; m = male.
[c] 1^{01} = *Suvar* $(2)1^{01}$; mapping and viability from REUTER, DORN and HOFFMANN (1982).

	204	209	207	210	213	214	215	1^{01}
204	.31 / .31	+	+	+	+	+	+	+
209		nd	+	+	+	+	+	+
207			0 / 0	.21 / .18	.25 / .26	.24 / .20	.26 / .26	.29 / .15
210				0 / 0	.24 / .11	.23 / .15	+	+
213					0 / 0	.22 / .03	+	.31 / .01
214						.16 / 0	+	.31 / .06
215							.22 / .16	+
1^{01}								nd

FIGURE 1.—Results of *inter se* complementation analysis involving the nine mutants in the 2L group, based on female infecundity. More than 100 *trans*-heterozygous progeny were generated for each cross and more than 50 females were examined per test. Viability of noncomplementing *trans*-heterozygotes and homozygotes are also presented as proportion of total offspring that were phenotypically Cy^+ (female and male data are give above and below, respectively); a value of 0.33 indicates normal viability. In all cases, survivors bearing noncomplementing mutations exhibited a red-brown eye colour (more severe in males) and the female survivors were infecund (failed to produce eggs upon testing). + = complementation nd = not determined (a stock containing a homozygously viable second chromosome bearing *Su(var)209* has not been constructed; for estimates of *Suvar(2)*1^{01} viability, see REUTER, DORN and HOFFMANN 1982).

RESULTS

Dominant variegation-suppression phenotypes: The effects of the nine *Su(var)* mutations from the 2L group on three different variegating rearrangements are summarized in Table 1 (columns 5–7). Most of the mutants strongly suppress all three variegators; however, there are exceptions. For example, *204* suppresses the w^{m4} and Sb^V phenotypes but only marginally affects bw^V, and *216* is highly sexually dimorphic with respect to suppression of w^{m4} and bw^V. (Because of the presence of the *Tft* marker on the *216*-bearing second chromosome, it was not possible to test the effects of *216* on Sb^V). Finally, although 1^{01} suppresses both w^{m4} and Sb^V, it actually enhances the variegation phenotype of bw^V. Despite these minor exceptions, the data suggest that the *Su(var)* mutants in this group affect PEV in a general fashion.

Viability and sterility phenotypes: In our previous paper we reported that six of seven *Su(var)* mutants from the 2L group (excluding the homozygously viable *209*) are lethal as homozygotes (SINCLAIR, MOTTUS and GRIGLIATTI 1983). This conclusion was based on tests for cosegregation of lethal and *Su(var)* phenotypes. In the present study we tested the viability of the newly mapped strain, *204*, and have reexamined the viability of the others using recombinant second chromosomes that lack the *b*, *lt* and *rl* markers carried by the original *Su(var)*-bearing chromosomes. We have found that homozygosity for *207*, *210*, *213* and *216* is completely lethal in both sexes, whereas *214* and *215* homozygotes exhibit semilethality, and *204* and *209* homozygotes are viable (Table 1, column 3; see Figure 1). Interestingly, *214/214* and *215/215* females produce no eggs and exhibit a red/brown eye color (this phenotype is also seen in surviving *215/215* males). On the other hand, homozygous *204* and *209* females are fecund and neither of these genotypes display the abnormal eye phenotype. None of the mutations affects male fertility. However, all of the *Df2*/mutant combinations, except *Df2/209*, exhibit male lethality and/or female infecundity (see below).

Although initial recombination mapping studies supported the conclusion that the lethal and *Su(var)* phenotypes cosegregated, the results of more extensive mapping experiments suggest that both the *207* and *216* chromosomes contained closely linked second site lethals (see below). Even after removal of the secondary lesion, the *207* chromosome remains homozygous lethal. We were unable to isolate *216* recombinant chromosomes that lacked the second lesion; however, subsequent genetic and molecular analysis revealed that it is an allele of the *cdc2Dm* gene, cloned recently by LEHNER and O'FARRELL (1990) and JIMENEZ *et al.* (1990) (N. J. CLEGG, I. WHITEHEAD and T. A. GRIGLIATTI, manuscript in preparation).

Many of the *Su(var)* mutants exhibit dominant

TABLE 2

Maternal-effect expression of *Su(var)* mutants from 2L group

	Percent Oregon-R pigment levels							
	Maternal cross[a]				Paternal cross[b]			
	CyO/+		*Su(var)*/+		*CyO*/+		*Su(var)*/+	
Strain	m	f	m	f	m	f	m	f
204	65 ± 5	52 ± 5	75 ± 5	98 ± 6	19 ± 6	36 ± 5	36 ± 3	86 ± 9
207	31 ± 4	51 ± 5	95 ± 5	127 ± 10	17 ± 1	34 ± 5	81 ± 7	111 ± 10
209	95 ± 4	91 ± 4	94 ± 4	101 ± 6	13 ± 2	26 ± 7	81 ± 5	86 ± 7
210	35 ± 2	32 ± 2	85 ± 4	96 ± 5	19 ± 2	48 ± 3	70 ± 3	99 ± 8
213	27 ± 3	22 ± 4	96 ± 4	99 ± 4	9 ± 1	14 ± 2	67 ± 6	93 ± 8
214	46 ± 6	29 ± 6	99 ± 4	98 ± 6	26 ± 4	32 ± 3	87 ± 11	105 ± 7
215	48 ± 6	46 ± 8	99 ± 5	105 ± 3	15 ± 2	24 ± 4	72 ± 6	95 ± 8
216	64 ± 8	64 ± 11	108 ± 8	123 ± 6	21 ± 4	21 ± 4	87 ± 5	100 ± 7

[a] Maternal cross = w^{m4}/w^{m4}; *Su(var)/CyO* females (f) × w^{m4}/Y males (m).
[b] Paternal cross = w^{m4}/Y; *Su(var)CyO* males × w^{m4}/w^{m4} females. Experiment performed at 29°.

maternal-effect phenotypes: SPOFFORD (1967, 1969) reported that a recessive *suppressor-of-variegation* mutation displayed a strong maternal effect phenotype with respect to PEV. Similar phenotypes are characteristic of some third chromosome *Su(var)* mutants (HARDEN 1984). This observation is consistent with the notion that these *Su(var)*$^+$ products are required early in development. We have examined eight mutants from the 2L group for analogous traits (Table 2). We used w^{m4}, since this rearrangement by itself does not exhibit a perceptible maternal-effect on w^+ expression (D. SINCLAIR, unpublished observations). The experiment was performed at 29° in order to facilitate the identification of more modest maternal effects. The data indicate that *204*, *209*, *215* and *216* had moderate to strong maternal-effect phenotypes (as evidenced by elevated pigment levels in non-suppressor-bearing offspring of *Su(var)* females). The strong phenotype of *209* was observed regardless of rearing temperature (data not shown). In contrast, *207* and *214* exhibited only modest maternal-effect phenotypes and *210* and *213* displayed none. The absence of a sexually dimorphic phenotype for *216* in the paternal cross (see Table 1) is likely due to the higher culture temperature.

Effects of reductions in cellular heterochromatin on the dominant *Su(var)* phenotype: Removal of heterochromatic elements or segments of heterochromatin from the genome can dramatically enhance the variegation phenotype (SPOFFORD 1976). We previously reported that the suppression phenotypes of several *Su(var)* mutants in the 2L group are essentially abolished in X/O males (SINCLAIR, MOTTUS and GRIGLIATTI 1983). We have extended this analysis to include all nine mutants (Table 3, columns 2 and 3). With the exception of l^{01}, all of the mutants are clearly sensitive to loss of the *Y* chromosome.

We next tested the effects of *Df(2R)MS2*^{10} on the phenotypes of these mutants. This deficiency lacks nearly all of the centromeric heterochromatin in 2R (LINDSLEY and GRELL 1968; HILLIKER and HOLM 1975). Our data suggest that *204*, *213*, *214*, *215* and *216* are markedly sensitive to removal of the 2R block, whereas *210* and l^{01} are affected only slightly, and *207* and *209* are relatively insensitive (Table 3, columns 4 and 5).

Effects of alterations in cellular heterochromatin on the *Su(var)* lethal phenotype: REUTER and coworkers reported that the severity of the semilethal phenotypes of *Suvar(2)1* alleles is dependent on the amount of heterochromatin in the genome (REUTER, DORN and HOFFMAN 1982). Extra heterochromatin exacerbates the phenotypes, whereas removal of heterochromatin has the opposite effect. We have examined the effects of addition or removal of the *Y* chromosome on the hemizygous lethal phenotypes of six mutants from the 2L group (Table 4). For five of the mutants, *207*, *210*, *213*, *215* and l^{01}, the extent of hemizygous lethality was dramatically enhanced in the presence of extra heterochromatin (*i.e.*, $\widehat{XX}/Y$ females), whereas lethality did not occur in the absence of the *Y* (*i.e.*, in X/O males). On the other hand, addition of a supernumerary *Y* chromosome had a more modest effect on survival of *204/Df2* females. The addition of a *Y* chromosome also appears to reduce the viability of the balancer-bearing females in each of the experimental crosses (compare the $\widehat{XX}/Y$; *Cy vs.* X/O; *Cy* data in columns 3 and 5, respectively). Finally, we have found that $\widehat{XX}/Y$; *Df2/209* females are viable and fecund (data not shown), suggesting that *209* is either an allele of a nonessential gene located within the segment deleted by *Df2*, or a hypomorphic allele of an essential gene located elsewhere on the chromosome.

TABLE 3

Effects of removal of cellular heterochromatin on expression of *Su(var)* mutants from 2L group

	Percent control pigment levels			
	Y constitution[b]		Autosomal constitution[c]	
Mutant[a]	w^{m4}/O	w^{m4}/*Y*	*Df(2R) MS2*^{10}/ *Su(var)*	*SM1,Cy*/ *Su(var)*
204	6 ± 1	65 ± 4	20 ± 1	76 ± 3
207	41 ± 2	73 ± 5	91 ± 2	99 ± 1
209	2 ± 1	70 ± 4	70 ± 4	
210	21 ± 3	82 ± 4	61 ± 2	96 ± 2
213	9 ± 1	89 ± 2	24 ± 3	81 ± 1
214	7 ± 1	103 ± 4	32 ± 3	81 ± 1
215	10 ± 1	73 ± 7	25 ± 1	84 ± 5
216	15 ± 1	99 ± 4	37 ± 1	93 ± 3
1^{01}	76 ± 4	82 ± 3	47 ± 4	82 ± 3
w^{m4}	3 ± 1	10 ± 2	1 ± 0	10 ± 3

[a] 1^{01} and *209* chromosomes bear no markers; *216* chromosome bears *Sp* and *Tft*; all other *Su(var)* chromosomes bear *b*, *lt* and *rl*.

[b] Male progeny (w^{m4}/O; *Su(var)*/+ or w^{m4}/*Y*; *Su(var)*/+) from w^{m4}/*Y*; *Su(var)*/*CyO* males × *C(1)RM,pn*/O or w^{m4}/w^{m4} females; pigment data calculated relative to Oregon-R.

[c] Male progeny (w^{m4}/*Y*; *Su(var)*/*Df(2R)MS2*^{10} or w^{m4}/*Y*; *Su(var)*/*SM1,Cy* from w^{m4}/w^{m4}; *Su(var)*/*CyO* females × +/*Y*; *SM1,Cy*/*Df(2R)MS2*^{10} males; experimental pigment data calculated relative to Oregon-R for 1^{01}, *209* and *216* and relative to *b lt rl*/*Df(2R)MS2*^{10} for the others; control data calculated relative to Oregon-R.

TABLE 4

Effects of alterations in amount of cellular heterochromatin on viability of *Su(var)* mutants from 2L group

	Number of progeny							
	Experimental[b]				Control[c]			
	$\hat{X}\hat{X}/Y$		*X*/O		*X*/*X*		*X*/*Y*	
Mutant[a]	*Cy*$^+$	*Cy*	*Cy*$^+$	*Cy*	*Cy*$^+$	*Cy*	*Cy*$^+$	*Cy*
204	30	126	131	171	86	158	70	127
207	0	140	153	195	92	265	2	208
215	7	52	68	92	133	283	68	225
210	0	37	76	105	100	139	0	109
213	1	177	124	307	57	195	0	153
1^{01}	0	123	140	317	16	42	0	31

[a] *210* and *215* were linked to the *b*, *lt* and *rl* markers, whereas *213* was linked to *b* and *Tft*; the 1^{01}-bearing chromosome was unmarked.

[b] Experimental cross: *C(1)RM, pn*/O; *Su(var)*/*CyO* females × *X*/*Y*; *Df2/SM1,Cy* males.

[c] Control cross: *X*/*X*; *Su(var)*/*CyO* females × *Df2/SM1,Cy* males. *Cy*$^+$ = *Df2/Su(var)*. *Cy* = *Df2/CyO* or *Su(var)*/*SM1,Cy*.

Several of the *Su(var)* mutants are butyrate-sensitive: Sublethal concentrations of sodium butyrate can suppress PEV in Drosophila (MOTTUS, REEVES and GRIGLIATTI 1980). REUTER, DORN and HOFFMANN (1982) reported that *Suvar(2)1* alleles exhibit reduced viability when raised on medium containing this chemical. We have tested *204*, *207*, *209* and *215* for sensitivity to butyrate (Table 5). Our results show that, with the exception of *209*, all of these strains appear to be perceptibly sensitive. This phenotype was usually, though not exclusively, observed in males.

TABLE 5

Effects of sodium butyrate on survival of specific *Su(var)* mutants from 2L group

	Percent survival[a]							
	Control medium[b]				Butyrate medium[b]			
	Cy		*Cy*$^+$		*Cy*		*Cy*$^+$	
Mutant	m	f	m	f	m	f	m	f
204	79	74	108	91	79	100	56	105
207	126	101	84	102	83	126	5	44
209	114	106	82	87	90	102	77	76
215	123	97	79	76	63	101	13	68

[a] Percent survival = raw % calculated as [observed no. adults/expected number adults] × 100; each value was then normalized according to viability of w^{m4} controls.

[b] Cross: *Su(var)*/*CyO* males × *Df2/SM1,Cy* females. Viability of w^{m4} controls (% survival = [no. eggs/no. adults] × 100) = 77 and 89% for untreated males and females, respectively; 82 and 88% for butyrate-treated males and females, respectively. No. embryos per culture ranged from 435 to 595. *Cy* = *Df2/CyO* or *Su(var)*/*Cy,SM1*; *Cy*$^+$ = *Su(var)*/*Df2*. m = males; f = females.

***Inter se* complementation analysis:** Previously, we reported that the mutants in this group defined three or four separate genes (SINCLAIR, MOTTUS and GRIGLIATTI 1983). This pattern of complementation was based on lethality or semilethality of *trans*-heterozygotes. However, since the mutants were induced with EMS and also because the mutagenized chromosomes contained the heterochromatic mutations, *light* (*lt*) and *rolled* (*rl*), we were concerned that the previous pattern of complementation might in part reflect nonspecific lethality. Moreover, we have observed that females hemizygous for *Su(var)* mutants are usually infecund (see below). For these reasons, we repeated the *inter se* complementation analysis using recombinant chromosomes that lacked *lt* and *rl* mutations and we tested female *trans*-heterozygotes for fecundity. Reciprocal crosses were performed for each test and since the results were similar, the data were pooled. Consistent with our previous results, we found that only *204* and *209* complemented all of the other mutants with respect to female fecundity (Figure 1). Most of the other combinations were female infecund indicating extensive noncomplementation. In addition, some *trans*-heterozygotes (*e.g.*, *213/214*, *213*/1^{01} and *214*/1^{01}) exhibited markedly reduced viability in males. Interestingly, flies bearing noncomplementing mutations exhibited a red-brown eye color and less frequently, a wings-held-out phenotype (not shown). These traits were also observed in surviving homozygotes and were most striking in males. Moreover, they were expressed even in the absence of w^{m4}. Since we were unable to obtain a *216* recombinant chromosome that lacked the *cdc2* lesion, we have not included the data from the *216* crosses. However, in separate com-

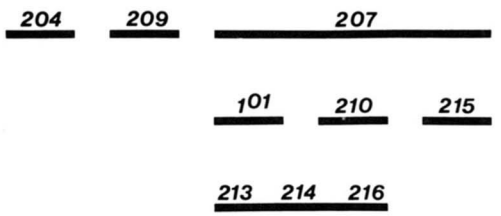

FIGURE 2.—A revised complementation map of the nine dominant *Su(var)* mutations in the 2L group based on female infecundity. Left-right orientation is arbitrary.

plementation tests between a strain carrying both the *216* second chromosome and an *X*-linked insertion of the *cdc2*$^+$ gene (generously provided by C. LEHNER), and the *207* and *214* strains, all of the diagnostic double mutant females bearing the insert were infecund and displayed the eye color and wing phenotypes (N. CLEGG, data not shown).

A revised complementation map for the *Su(var)* mutants, based on female infecundity, is shown in Figure 2. Our interpretation is that the nine *Su(var)* mutants define three separate genes: two genes *204* and *209*, each defined by a single allele and a third gene (hereafter referred to as *Suvar(2)1*) with seven alleles. The inclusion of *Su(var)216* as an allele of this gene is based on the results of the two complementation crosses mentioned above. The argument that *204* represents a separate gene is fairly compelling, since it is female infecund when heterozygous with certain section 31 deficiencies (see below), yet it complements fully all of the other mutants. Given that *209* displays no recessive lethal or infecundity phenotypes, its complementation pattern may not be surprising. However, our contention that it identifies a separate *Su(var)* gene is also supported by the finding that, unlike representative alleles of the other two loci, the *209* mutation is not butyrate-sensitive (Table 5).

Cytological localization of the *Su(var)* mutants: A preliminary mapping experiment using *J* as the reference marker, placed *214* (the suppressor phenotype) 0.3 map unit distal to this locus (data not shown). Since *J* is located at map position 41 in 2L, it seemed likely that 2L deletions isolated as revertants of *J* (SANDLER 1977; SALAS and LENGYEL 1984), as well as *Dp(2;2)Mdh3*, would be useful for localizing the *Su(var)* mutants (Figure 3). It should be emphasized that although rearrangement breakpoints in 31A and 31F-32F can be placed with reasonable accuracy, the diffuse banding pattern in 31B-31E makes positioning of breakpoints in this interval more difficult.

The approximate breakpoints of *Df2*, *Df27*, *Df39*, have been reported previously (MANGE and SANDLER 1973; SANDLER 1977). Our observations differ from the earlier reports in two major respects. First, despite the ambiguity concerning the exact position of the distal breakpoint of *Df39*, we believe that it does not extend beyond the 31B/C boundary. Therefore, it

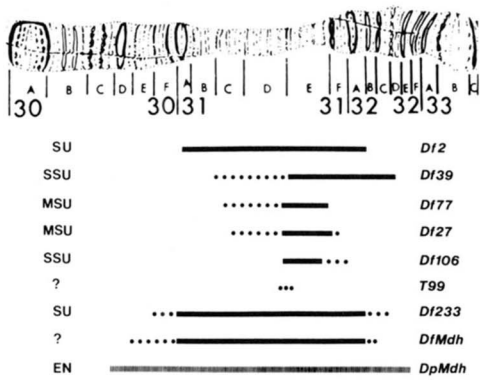

FIGURE 3.—A map of sections 30–32 of chromosome *2*, with locations of relevant rearrangements shown below. The deficiencies (*J* series and *Df(2L)Mdh2*) are represented by solid bars, with cytological uncertainties indicated by dots. The approximate position of *T(Y;2)J99* (*T99*) and the extent of *Dp(2;2)Mdh2* (dashed line) are also shown. The effects of various rearrangements on the w^{m4} phenotype are summarized on the left (see Table 6 and text): SU = strong suppression; MSU = moderate suppression; SSU = slight suppression; EN = enhancement; ? = not tested.

seems unlikely that the *Minute* (*M*) phenotype associated with this chromosome is due to the inclusion of *M(2)fs* within the limits of the deletion (see SANDLER 1977). This suggests that either another *M* locus exists within the deleted segment, or that the *Df39* chromosome carries a second-site *M* lesion. Second, we have detected an additional 2L deletion within polytene region 22 on the *Df2* chromosome (data not shown). The breakpoints of the other *J* deficiencies, *Df106*, *Df77* and *Df233*, as well as that of *T99*, are also shown in Figure 3. *Df233* is a complex rearrangement involving a deletion spanning section 31 in association with a translocation between the second and third chromosomes (data not shown). Since *Df233*/+ females display a *M* phenotype and are sterile (*i.e.*, they lay eggs that fail to develop, data not shown), it is likely that the *M(2)fs* gene is located within the deleted segment. *Df77* and *Df106* remove smaller portions of section 31; *Df77* minimally deletes all of 31E and may extend distally into 31C, whereas *Df106* deletes most of 31E and appears to extend into the 31F segment. The breakpoint of *T99* indicates that the *J* locus is located close to the distal edge of 31E.

Several studies have identified chromosomal regions containing haplo-abnormal *Su(var)* loci in Drosophila (HENIKOFF 1979; REUTER and SZIDONYA 1983; REUTER *et al.* 1986; LOCKE, KOTARSKI and TARTOF 1988; WUSTMANN *et al.* 1989). We have examined the 31–32 region of 2L for the presence of analogous loci by testing the 2L deletions and *Dp(2;2)Mdh3* for their effects on the w^{m4} phenotype. The results of this experiment (Table 6 and summarized on the left in Figure 3) indicate that most of the deletions cause some suppression of w^{m4}. We arbitrarily designate this effect as slight (23–27%) for *Df39* and *Df106*, to moderate (40–60%) for *Df27* and *Df77*,

TABLE 6

Effects of various *Jammed*-derived deficiencies and *Dp(2;2)Mdh3* on w^{m4} variegation

Second chromosome genotype of male[a]	Percent Oregon-R pigment levels	Effects on w^{m4}
+/+	3 ± 1	
Df2/+	81 ± 4	Full suppression
Df233/+	74 ± 1	Full suppression
Df77/+	59 ± 2	Moderate suppression
Df27/+	42 ± 3	Moderate suppression
Df106/+	27 ± 1	Slight suppression
Df39/+	23 ± 1	Slight suppression
Dp(2;2)Mdh3/+	0	Enhancement

[a] Sex chromosome constitution of males = w^{m4}/Y. Experiment performed at 25°.

to strong (70–80%) for both *Df233* and *Df2*. Despite the rather surprising fact that *Df39* only modestly suppresses w^{m4}, these data suggest there are at least two dose-dependent *Su(var)* loci in section 31, one defined by the overlap between *Df77* and *Df27* and a second, more distal locus deleted by *Df2* and *Df233*.

Triplo-abnormal effects have also been reported for many dose-dependent modifiers of position-effect variegation (REUTER and SZIDONYA 1983; LOCKE, KOTARSKI and TARTOF 1988; WUSTMANN *et al.* 1989). *Dp(2;2)Mdh3*, which is duplicated for 30D-32E/F, strongly enhances the w^{m4} phenotype (Table 6, last row), as well as those of w^{mMc} and w^{m51b}. It also enhances variegation of the *roughest* (*rst*) gene (distal to *w*) in flies bearing these rearrangements (SINCLAIR, LLOYD and GRIGLIATTI 1989; T. A. GRIGLIATTI, unpublished observations). Consistent with previous findings regarding PEV (SPOFFORD 1976), we found that enhanced *rst* variegation was restricted to clones of ommatidia in which the *w* gene was inactive (data not shown). Thus, we conclude that a duplication of one or more *Su(var)* loci in this region promotes variegation and it does so in a polarized fashion.

We next examined the w^{m4} phenotypes of heterozygous combinations between the mutants and *Dp(2;2)Mdh3* (Table 7). In all cases, the higher pigment levels exhibited by the *Su(var)*-bearing control males (column 3), were markedly reduced in their *Su(var)/Dp* counterparts (column 2). These deficiency and duplication data are consistent with the view that all eight *Su(var)* mutations map within the 30D–32E/F interval. We believe that the alternative possibility, namely that the duplication does not contain the $Su(var)^+$ loci, but is merely counteracting the *Su(var)* phenotypes, is unlikely. We have observed some counteracting effects in a minority of crosses involving the *Dp* and a large number of second and third chromosome *Su(var)* mutants; however, pigment levels in *Dp/Su(var)* or *Dp*/+; *Su(var)*/+ flies always exceeded 35% of wild-type controls (SINCLAIR, LLOYD and GRIGLIATTI 1989 and data not shown). This is in marked contrast to the data shown here, where the *Su(var)/Dp* pigment levels were as low as that of the w^{m4} controls.

TABLE 7

Effects of *Dp(2;2)Mdh3* on expression of *Su(var)* mutants from 2L group

Mutant	Percent Oregon-R pigment levels[a]	
	Su(var)/Dp	*Su(var)*/+
204	5 ± 1	63 ± 4
207	3 ± 1	73 ± 5
209	8 ± 1	70 ± 5
210	16 ± 1	82 ± 5
213	1 ± 0	89 ± 2
214	1 ± 0	103 ± 4
215	4 ± 1	73 ± 7
216	2 ± 0	38 ± 5

[a] w^{m4}/Y; *Su(var)/Dp* data are from crosses between w^{m4}/w^{m4}; *Su(var)/CyO* females and +/Y; *Dp(2;2)Mdh3/SM1, Cy* males. w^{m4}/Y; *Su(var)*/+ data are from Table 1. w^{m4} control = 10 ± 4.

TABLE 8

Summary of results of mapping experiment involving 2L deletions and *Su(var)* mutants from 2L group

Deletion	*Su(var)* mutations[a]								
	204	*207*	*209*	*210*	*213*	*214*	*215*	*216*	1^{01}
Df2	v/i	v/i	v/f	l/i	l/i	l/i	v/i	l/l	l/i
Df39	v/i	l/l	np	v/f	v/f	v/f	v/f	l/l	v/f
Df77	v/f	v/f	v/f	v/f	v/f	v/f	v/f	l/l	v/f
Df27	v/f	v/f	v/f	v/f	v/f	v/f	v/f	l/l	v/f
Df106	v/f	v/f	v/f	v/f	v/f	v/f	v/f	l/l	v/f
Df233	v/i	l/l	v/f	l/l	l/l	l/l	l/i	l/l	np

[a] In most cases, data summary = male viability/female fecundity (note that all of the *216/Df* combinations were completely lethal in both sexes). Range of progeny examined per test = 60–1100. v, viable; l, lethal; f, females were fecund; i, females were infecund (no eggs produced). np = cross not performed.

We next used the deletions in an attempt to localize the *Su(var)* mutants in section 31. Results of relevant crosses are summarized in Table 8. In most cases, viability or inviability pertains to male data only. Two observations from the *Df2* data (row 1) suggest that seven of the mutants map within the limits of this deletion: (i) heterozygous combinations between *Df2* and *210*, *213*, *214* and 1^{01} are lethal in males (note that the *Df2/216* combination is lethal in both sexes due to the presence of the *cdc2* lesion; see below); (ii) excluding *209* and *216*, all *Df2/Su(var)* females are infecund, *i.e.*, they produce no eggs when mated.

From the remaining crosses, it can be seen that *210*, *213*, *214*, *215* and 1^{01} are located distally within the 31A–D region. This agrees with the contention that all five mutations are alleles of a single *Su(var)* gene. Furthermore, the infecundity of *Df39/204* females supports our argument that *204* is a separate *Su(var)* gene located at a more proximal position in 31C-D

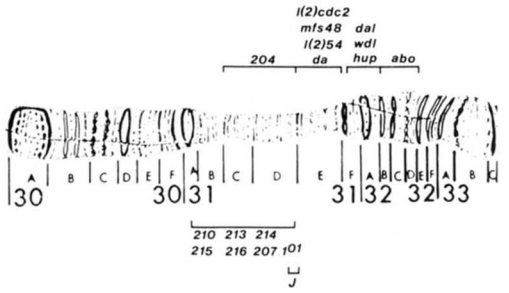

FIGURE 4.—A map of sections 30–32 of chromosome *2* showing the cytological positions of dominant *Su(var)* mutations, *J* and a series of recessive maternal-effect or lethal mutations. The placement of *207* and *216* is based on their failure to complement *Suvar(2)1*^{01}, as well as deficiency data (see text). Due to uncertainty about deficiency breakpoints, it is not possible to position *204* precisely relative to the *Suvar(2)1* gene. The position of the second site lethal allele of *cdc2* borne by the *216* chromosome is also shown.

(we have recombinationally mapped the *204* suppression phenotype to the left of *J*, data not shown).

Although the *207* and *216* deficiency data are at odds with the conclusion that they are alleles of *Suvar(2)1*, they are consistent with our finding that both mutant chromosomes contain closely linked second site lethals. It is evident that the *cdc2* lesion on the *216* chromosome maps within 31E and this agrees with the *in situ* localization of the cloned gene (LEHNER and O'FARRELL 1990; JIMENEZ *et al.* 1990). Furthermore, using the *216* strain containing the *X*-linked insert of *cdc2*$^+$, N. CLEGG has deficiency mapped the female infecundity phenotype of *Su(var)216* to the site of the *Suvar(2)1* locus in 31A–D (data not shown). We have recombinationally mapped the second site lesion associated with *207* to the right of *J* (data not shown), but its identity remains undefined. However, given the position of the secondary lesion, the deficiency data are clearly consistent with the inclusion of *207* in the *Suvar(2)1* locus.

The combined results of the cytogenetic and complementation analyses allowed us to construct a chromosomal map of the mutations from this study (Figure 4). We conclude that the multi-allelic *Suvar(2)1* gene is located in 31A–D and that the separate *204* gene is located in 31C-D. Since we cannot unequivocally position the *209* gene, it is not included in the map. However, the position of the *cdc2* gene, as defined by the second site mutation on the *216* chromosome, is shown.

Other genes located within the 31–32A interval: Several other genes that map within the 31–32A region have been identified. These include the maternal-effect genes *da* (which also has essential zygotic functions; reviewed by CLINE 1989), *abo*, *hup*, *wdl*, and *dal* (these four mutants cause female semisterility when homozygous or hemizygous), and the essential genes *mfs48* and *l(2)54* (SANDLER 1977). Mutant alleles of the maternal-effect genes are of particular interest since, like the *Su(var)* mutations, they are sensitive to alterations in the amount of heterochromatin in the genome. Moreover, *da* encodes a member of the helix-loop-helix class of DNA-binding proteins (MURRE, MCCAW and BALTIMORE 1989). Although the nature of the *dal*$^+$ product is unknown, SULLIVAN, MINDEN and ALBERTS (1990) report that this gene affects centrosome behavior during embryonic cell division.

TABLE 9

Summary of complementation data for crosses between 2L deletions and specific mutations that map to 31–32 interval

	Mutations						
Deletion	*da*	*mfs48*	*l(2)54*	*wdl*	*hup*	*dal*	*abo*
Df2	l	l	l	sl[a]	v	v	v
Df77	l	l	l	v	v	v	v
Df106	l	l	l	v	v	v	v
Df27	l	l	l	v	v	v	v
Df39	l	l	l	l	l	l	sl
Df233	l	l	l	l	l	sl	v

[a] *wdl/Df2* flies display a *wavoid-like* wing phenotype (SANDLER 1977). Crosses = *Df/Cy* (*Cy* = *SM1* or *SM5*) females × mutant/*CyO* males. Range of no. of progeny scored per test = 89–1258. v, viable; l, lethal; sl, semilethal (<30% expected).

SANDLER (1977) cytologically mapped *da*, *mfs48* and *l(2)54* within 31B–F and *wdl*, *hup*, *dal* and *abo* within 32A–E. In order to characterize more fully the functional organization of this region of the genome, we have extended the deletion mapping of these mutants (Table 9). Our data position *da*, *mfs48* and *l(2)54* in 31E (Figure 4). The positioning of *da* agrees with the *in situ* localization of the cloned gene (CAUDY *et al.* 1988; CRONMILLER, SCHEDL and CLINE 1988). The semilethality and *wavoid*-like phenotype of *Df2/wdl* heterozygotes suggest that the *wdl* gene is located within the 31F–32A interval. Indeed, fertility tests involving females heterozygous for *wdl*, *hup*, or *dal* and the various deficiencies have revealed that all three genes are located in this interval (data not shown; Figure 4). *abo* exhibits a semisterile phenotype only when heterozygous with *Df39* (data not shown), indicating that this gene is located within the 32A4–32E interval (Figure 4). This is consistent with the recent molecular study suggesting that the *abo* gene may reside in 32E (LAVORGNA *et al.* 1989).

Recessive suppressors of PEV in 31E-32A: The relatively close proximity of *da*, *mfs48* and *l(2)54* to the dominant *Su(var)* genes in section 31, prompted us to examine the effects of all five mutants on PEV. Although none of them consistently affects the expression of w^{m4} in a dominant fashion (data not shown), both *mfs48* and *wdl* suppress w^{m4} when homozygous (Table 10). Due to reduced viability of *mfs48* and *wdl*, it is not possible to recombinationally map the suppression phenotypes. However, rare *mfs48/Df106* survivors also exhibit clear suppression of w^{m4} (data not

TABLE 10

Effects of various recessive mutations from 31E–32A region on w^{m4} variegation

Strain[a]	Percent Oregon-R pigment levels	
	Males	Females
mfs48/mfs48	33 ± 1	86 ± 3
wdl/wdl	44 ± 3	82 ± 4
hup/hup	10 ± 1	29 ± 2
da/da	37 ± 1	25 ± 4
dal/dal	20 ± 2	31 ± 2
w^{m4} controls[b]	16 ± 2	16 ± 1

[a] Flies assayed from w^{m4}; mutant/*CyO* stock cultures.
[b] Controls: w^{m4}/Y; *+/Cy,SM1* males and w^{m4}/w^{m4}; *+/Cy,SM1* females from cross between w^{m4}/w^{m4} females and *wdl/Cy,SM1* males.

shown). Interestingly, the suppressor phenotypes of both mutants, like that of *216*, are sexually dimorphic. While the other mutants also appear to cause somewhat elevated pigment levels in a w^{m4} background in one or both sexes (Table 10, note especially *da* males), these effects were much less striking than those observed for homozygous *mfs48* and *wdl* females. Thus, we conclude that there are at least two genes in 31E–32A capable of recessive suppression of PEV.

DISCUSSION

In this paper, we present a detailed phenotypic and cytogenetic analysis of a group of mutations that we had localized to a discrete region on the left arm of chromosome two (SINCLAIR, MOTTUS and GRIGLIATTI 1983). Our results suggest that the nine mutants comprising this group define three separate *Su(var)* genes; two of these genes, including *Suvar(2)1* identified by REUTER and co-workers, are located in section 31 of 2L, a region containing one or more *Su(var)* loci that are both haplo- and triplo-abnormal.

Mutant alleles of the three genes are reasonably strong and general with respect to PEV. The fact that some of them differ in terms of their effects on different variegators may be attributable to intrinsic properties of the variegators or the lesions. All three genes exhibit maternal-effect suppression of w^{m4}, indicating that the $Su(var)^+$ products are required early in development. SZABAD, REUTER and SCHROEDER (1988) have drawn a similar inference from their study of $Suvar(2)1^{01}$. Surprisingly, not all of the *Suvar(2)1* alleles in our collection display maternal effects. Mutations in two of the three genes, *Suvar(2)1* and *204*, affect viability and female fecundity, indicating that the respective wild-type products have important roles in the cell. Moreover, both genes exhibit butyrate sensitivity. REUTER, DORN and HOFFMANN (1982) report analogous findings for other alleles of the *Suvar(2)1* gene. In contrast, the *209* gene appears to be nonessential and the single mutant allele is not sensitive to butyrate. However, we cannot completely exclude the possibility that *209* is a weak hypomorphic allele of an essential *Su(var)* gene located outside section 31. Finally, an especially striking property of the mutants is their sensitivity to the amount of heterochromatin in the genome. It should be emphasized that, in the case of the *Su(var)* lethal phenotype, this effect is unrelated to PEV, since $\widehat{XX}/Y$; *Df2/Su(var)* females bear no variegating rearrangement. Therefore, it must reflect more extreme disruption of the normal cellular functions of the *Su(var)* products caused by additional heterochromatin.

The existence of haplo-insufficient *Su(var)* loci in section 31, together with our finding that a duplication for this region essentially abolishes the suppressor phenotypes of all of the mutants, is consistent with the proposal that these are loss of function lesions. On the other hand, although *Suvar(2)1/Df2* flies are phenotypically normal (but female infecund), *trans*-heterozygotes involving different alleles, as well as surviving homozygotes for weaker alleles, exhibit red-brown eye colour and wings-held-out phenotypes. More recent work indicates that the eye colour phenotype is due to reduced expression of the heterochromatic *light* (*lt*) gene (N. J. CLEGG, D. A. SINCLAIR and T. A. GRIGLIATTI, manuscript in preparation). These findings are difficult to reconcile with a simple loss-of-function hypothesis. Thus, we propose that while the *Suvar(2)1* gene is haplo-insufficient with respect to PEV, the alleles in our collection possess some antimorphic properties. We are interested in exploring this possibility further, since it suggests the existence of a novel class of suppressor locus that is distinguishable from previously defined dose-sensitive genes (LOCKE, KOTARSKI and TARTOF 1988; WUSTMANN *et al.* 1989). In contrast to the situation for the *Suvar(2)1* alleles, we believe that the *204* mutation is hypomorphic, since *204/204* females are fecund, whereas *204/Df2* females lay no eggs (data not shown). The nature of the *209* mutation remains undefined.

Several groups have proposed that *Su(var)* genes may encode specific types of heterochromatic NHPs (SPOFFORD 1976; HENIKOFF 1979; SINCLAIR, MOTTUS and GRIGLIATTI 1983; LOCKE, KOTARSKI and TARTOF 1988) and this has been confirmed for *Su(var)205* (EISSENBERG *et al.* 1990). We propose that both *Suvar(2)1* and *Su(var)204* be included in this category. The observation that one or both of these loci are haplo-abnormal with respect to PEV is consistent with this proposal, since this is a predicted property for NHP genes (MOORE, SINCLAIR and GRIGLIATTI 1983; WUSTMANN *et al.* 1989). Moreover, the ability of a variety of *Suvar(2)1* alleles to alter chromatin structure, another expected property of mutations in NHP genes, is well documented (REUTER, WERNER and

HOFFMANN 1982; DORN *et al.* 1986; HAYASHI *et al.* 1990).

The chromatin hypothesis can be extended to explain the lethality, female infecundity and eye color phenotypes exhibited by the *Su(var)* mutants. Since it has been suggested that specific types of chromatin architecture are required for expression of heterochromatic genes (WAKIMOTO and HEARN 1990), we propose that mutations in NHP loci can perturb this architecture, thereby disrupting the expression of genes such as *lt*. Decreased viability and female infecundity could result from the underexpression of one or more essential heterochromatic genes. Presumably, the conformational changes to chromatin caused by the *Su(var)* mutants could stem from either underproduction of key chromatin constituents, or assembly of abnormal NHPs.

A modified version of an earlier proposal (ZUCKERKANDL 1974) may explain the hypersensitivity of *Su(var)* mutants to the amount of genomic heterochromatin, within the context of the chromatin hypothesis. Thus, if it is assumed that heterochromatic elements such as the *Y* chromosome or 2R block possess abundant binding sites for chromatin multimers, subunits of which are encoded by certain *Su(var)* loci, then addition or removal of the elements could respectively decrease or increase the cellular level of the constituents (*i.e.*, the $Su(var)^+$ products). In principle, the former could produce a more extreme mutant phenotype *i.e.*, increased suppression of PEV in *Su(var)/+* flies, or increased lethality in *Su(var)/Df* or *Su(var)/Su(var)* individuals, whereas the latter would have the opposite effect. The degree of sensitivity exhibited by the mutants might vary according to the severity of the lesion, the cellular requirement for the gene product and the type and number of binding sites possessed by particular heterochromatic elements.

If the 209^+ product is truly nonessential then it may differ fundamentally from the products of the other two *Su(var)* genes. If it is also a structural component of heterochromatin, its apparent expendability might reflect significant functional overlap among some related NHPs and their genes. Alternatively, the 209^+ product may influence PEV via an entirely different mechanism. We are currently attempting to clone and characterize all three *Su(var)* genes in this region in order to resolve these questions.

The role of the *mfs48* and *wdl* genes with respect to PEV is unclear. The recessive mutants were originally isolated on the basis of strong semilethality over *Df39* (SANDLER 1977). Both genes affect fertility and the production of cuticular structures, as well as causing strong suppression of PEV. Interestingly, the maternal-effect lethality of *wdl* can be modified by altering the amount of heterochromatin in the genome (SANDLER 1977), suggesting some parallels between the bases of dominant and recessive suppression of PEV. Perhaps these genes encode chromatin assembly or modifying factors. We have recently cloned a *P* element-containing allele of *mfs48* (I. WHITEHEAD and N. J. CLEGG, unpublished data). The molecular analysis of this gene should provide insight into its role in PEV.

Previous studies, primarily using deficiency and duplication procedures, have provided estimates of PEV modifier loci in Drosophila ranging from as few as 20 or 30 (LOCKE, KOTARSKI and TARTOF 1988), to as many as 150 (WUSTMANN *et al.* 1989). Clearly, segmental aneuploidy is a useful tool for identifying regions of the genome that can influence PEV. However, we believe that the use of relatively large deficiencies and duplications to infer the existence of single modifying genes and to estimate numbers of such loci in the genome, may be problematical for several reasons. First, it has been shown that even relatively discrete autosomal regions may contain more than one locus that can modify PEV (*e.g.*, see HENIKOFF 1979; WUSTMANN *et al.* 1989 and the present study). This problem may be compounded if closely linked loci act antagonistically (see WUSTMANN *et al.* 1989). Second, some regions might contain recessive suppressors of PEV and obviously these would not be detected using the criterion of dose-dependence. Our finding that both *mfs48* and *wdl* suppress w^{m4}, together with the earlier work of SPOFFORD (1967, 1969), suggests that these types of genes are not rare. Third, removal or duplication of one or more "housekeeping" genes could have developmental consequences (*e.g.*, prolongation of development; see MICHAILIDIS, MURRAY and MARSHALL GRAVES 1988) that might indirectly modify PEV. Hence, only through fine-structure genetic and cytogenetic analyses of putative dose-dependent regions will it be possible to confirm the existence of *Su(var)* or *E(var)* loci and study their properties.

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