HISTONE GENE MULTIPLICITY AND POSITION EFFECT VARIEGATION IN *DROSOPHILA MELANOGASTER*

GERALD D. MOORE, DONALD A. SINCLAIR AND THOMAS A. GRIGLIATTI

Department of Zoology, The University of British Columbia, Vancouver, British Columbia, Canada V6T 2A9

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ABSTRACT

The histone genes of wild-type *Drosophila melanogaster* are reiterated 100–150 times per haploid genome and are located in the segment of chromosome 2 that corresponds to polytene bands 39D2-3 to E1-2. The influence of altered histone gene multiplicity on chromatin structure has been assayed by measuring modification of the gene inactivation associated with position effect variegation in genotypes bearing deletions of the 39D-E segment. The proportion of cells in which a variegating gene is active is increased in genotypes that are heterozygous for a deficiency that removes the histone gene complex. Deletions that remove segments adjacent to the histone gene complex have no effect on the expression of variegating genes. Suppression of position effect variegation associated with reduction of histone gene multiplicity applies to both *X*-linked and autosomal variegating genes. Position effects exerted by both autosomal and sex-chromosome heterochromatin were suppressible by deletions of the histone gene complex. The suppression was independent of the presence of the *Y* chromosome. A deficiency that deletes only the distal portion of the histone gene complex also has the ability to suppress position effect variegation. Duplication of the histone gene complex did not enhance position effect variegation. Deletion or duplication of the histone gene complex in the maternal genome had no effect on the extent of variegation in progeny whose histone gene multiplicity was normal. These results are discussed with respect to current knowledge of the organization of the histone gene complex and control of its expression.

THE histone genes of the sea urchin were among the first specific eukaryotic genes to be investigated biochemically (KEDES and BIRNSTIEL 1971). Subsequent molecular cloning and characterization of histone genes from various species have revealed common organizational features (reviewed by HENTSCHEL and BIRNSTIEL 1981). The five somatic histone proteins of Drosophila are encoded by a 5.0-kb sequence which is tandemly reiterated 100–150 times per haploid genome (LIFTON *et al.* 1977; CHERNYSHEV *et al.* 1980). This gene complex has been localized by *in situ* hybridization to a segment in the proximal region of the left arm of the second chromosome which corresponds to the salivary chromosome bands 39D2-3 to 39E1-2 (PARDUE *et al.* 1977). Since Drosophila exhibits rapid embryonic nuclear proliferation, it has been suggested that this pattern of gene organization facilitates the synthesis of large

quantities of histone for the packaging of rapidly replicating DNA (JACOB, MALACINSKI and BIRNSTIEL 1976). Sequences encoding single histone proteins have been reported to occur elsewhere in the genome; however, such "orphons" probably constitute only a small fraction of the potential histone coding capacity (CHILDS *et al.* 1981).

The relationship between the transcriptional activity of the histone genes and the cell cycle has been the subject of extensive scrutiny in a variety of species (for reviews, see BORUN 1975; KEDES 1979). The general conclusion arising from these investigations is that, with the exception of early embryogenesis and gametogenesis, the synthesis and translation of histone messenger RNA are tightly coupled to DNA replication. Differential expression of stage-specific subsets of histone genes is also observed during the development of diverse organisms. For example, in the sea urchin, development from cleavage to gastrula stages is accompanied by selective transcription of sequence variant histone genes, with a concomitant disappearance of histone mRNA of the earlier variant types (NEWROCK *et al.* 1977; GRUNSTEIN 1978). Similar, although less extensive, shifts of embryonic histone subtypes have been observed in Drosophila. In addition, Drosophila gametogenesis is characterized by histone replacement during spermiogenesis and the accumulation of maternal histone mRNA during oogenesis (PALMER, SNYDER and BLUMENFELD 1980; NEWROCK *et al.* 1978; ANDERSON and LENGYEL 1980).

In Drosophila, individuals that lack the 39D-E region entirely, die during embryogenesis; yet, flies that are hemizygous for this segment are viable and fertile and exhibit no diagnostically visible phenotype. However, definite phenotypic effects have been attributed to sublethal alterations in histone gene multiplicity. The most striking example of such an effect involves the phenomenon of position effect variegation. Position effect variegation usually occurs when a euchromatic gene is juxtaposed to a site within or near heterochromatin (for reviews, see BAKER 1968; SPOFFORD 1976). The heterochromatin is thought to influence the conformation of the adjacent euchromatin, leading to inactivation of genes in a subset of cells bearing the rearranged chromosome. This inactivation occurs early in development and is clonally propagated, resulting in a mosaic phenotype. Although the molecular nature of this effect is unclear, it is definitely not due to somatic loss of variegating gene sequences (HENIKOFF 1979a). The severity of the variegated phenotype has been positively correlated with the proportion of salivary gland cells in which the chromosomal site containing the variegating gene assumes a heterochromatin-like morphology (HARTMANN-GOLDSTEIN 1967). The heterochromatic components that cause this spreading effect remain unidentified. However, since, in mealybugs, inactivation of the paternally derived chromosomes is associated with an enriched histone content in those chromosomes (BERLOWITZ 1965), it occurred to us that histones may play an important role in heterochromatinization and, thus, in position effect variegation. Indeed, we succeeded in demonstrating that heterozygous deletions involving the histone gene region increased the activity of the variegating genes white-mottled-4 (w^{m4}) and Bar of Stone-Variegated (B^{SV}) (MOORE *et al.* 1979). Subsequently, MOTTUS, REEVES

and GRIGLIATTI (1980) established that chemical inhibitors of histone deacetylation (*e.g.*, sodium butyrate) enhance the activity of the w^+ gene in w^{m4} individuals. Taken together, these findings suggest that reductions in histone gene multiplicity, or alterations in the affinity of histones for DNA, may influence chromatin structure in the vicinity of genes subject to position effect variegation, resulting in a suppression of the inactivation of these genes. Although this interpretation may be somewhat simplistic, it provides a useful hypothesis for further experimentation.

This report addresses various questions concerning the nature and scope of the modification of position effect variegation associated with alterations in histone gene multiplicity. These include the following: (1) Can suppression of variegation be unequivocally correlated with loss of histone coding sequences? (2) Is the expression of variegating autosomal genes (which presumably are not subject to *X* chromosome dosage compensation) also increased by histone gene deletions? (3) What is the effect of a deletion that removes only a certain portion of the histone gene complex? (4) Does deletion-mediated suppression require the presence of *Y* heterochromatin? (5) Does a duplication of the histone gene complex affect variegation? and (6) Does the histone gene dosage of the maternal parent affect the expression of variegating genes in her euploid offspring?

MATERIALS AND METHODS

Culture conditions: Flies were reared in ½-pint milk bottles or 8-dram shell vials on a sucrose-cornmeal-agar medium, seeded with Baker's yeast. Tegosept (methyl-*p*-hydroxybenzoate) was included in the food as a mould inhibitor. To suppress bacterial growth, a combination of ampicillin and streptomycin, or ampicillin and tetracycline, was added (10 mg/liter, each). All crosses were performed at 22° (unless otherwise specified).

Mutant strains and special chromosomes: Description of the mutations and chromosomes used can be found in LINDSLEY and GRELL (1968), with the following exceptions: (1) Proximal (*2L*) deficiencies: *Df(2L)1*, *Df(2L)12*, *Df(2L)65*, *Df(2L)84* and *Df(2L)161* were generated in a screen for deficiencies of the dopa-decarboxylase locus performed by WRIGHT, HODGETTS and SHERALD (1976). *Df(2L)1*, *12*, *65* and *84* are X-irradiation-induced derivatives of a Tuft (*Tft*) lethal(2)74i chromosome, whereas *Df(2L)161* was induced by X rays on a cinnabar (*cn*) brown (*bw*) chromosome. *Df(2L)1*, *12*, *65*, *84* and *161* are deleted for the loci purple (*pr*), Bristle (*Bl*) and lethal (2) cryptocephal (*crc*). In addition, *Df(2L)161* lacks the Minute (2)H locus. Nine proximal *2L* mutations were induced with the mutagen triethylene melamine, on a black (*b*), purple (*pr*) cinnabar (*cn*) chromosome (SINCLAIR, MOORE and GRIGLIATTI 1980). Cytological analysis of these nine mutations (*l(2L)DS1-9*) revealed that four of them are associated with visible deficiencies. All of the visible deficiencies except *Df(2L)DS9* delete *l(2)crc*. A salivary chromosome map of the proximal (*2L*) deficiencies is presented in Figure 1.

(2) Duplication: *Dp(2;1)C239* is a segregant from the translocation strain *T(1;2)C239* in which the proximal *2L* region, bands 36C-39E inclusive, are duplicated and carried on the *X* chromosome. *Dp(2;1)C239w* carries the mutant allele white.

Genetic crosses: Crosses specific to the various experiments will be described in appropriate sections of RESULTS. All crosses were performed using 20–25 pairs of parents. F_1 male and/or female progeny were collected from a given cross and subjected to the respective analysis.

Eye pigment measurement: The fluorometric technique used for the quantification of eye drosopterin from single fly heads has been previously described (MOORE *et al.* 1979).

Bristle phenotype measurement: Bristle phenotype was quantified by either of two methods: (1) The dorsocentral and scutellar macrochaetes were observed by dissecting microscope and assigned

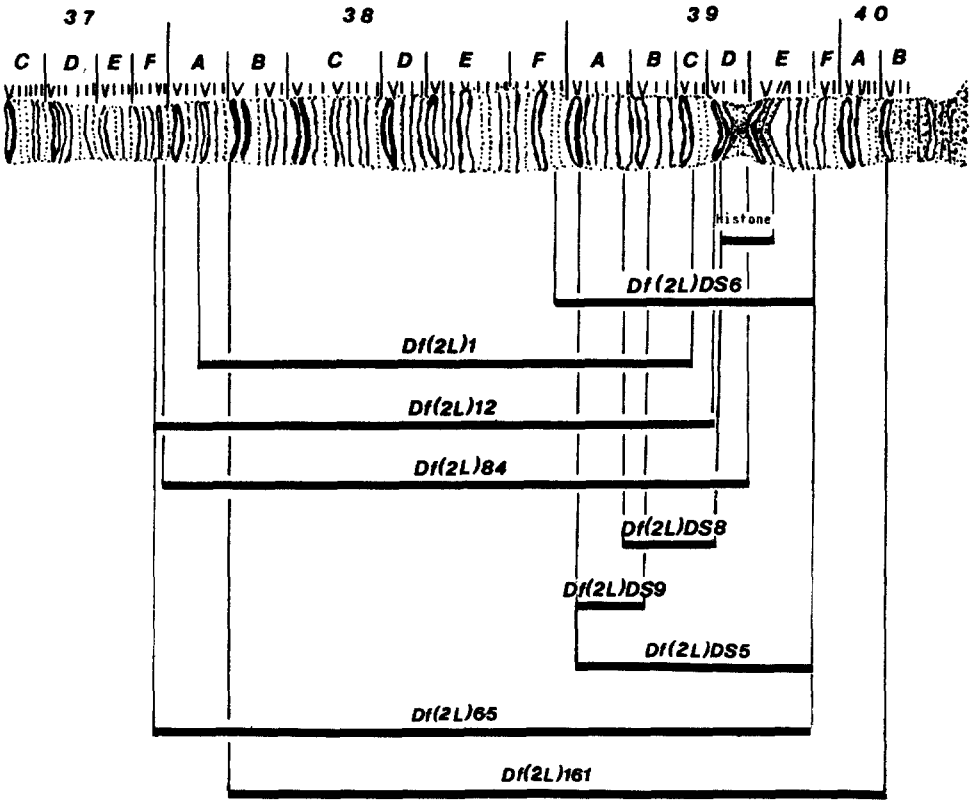

FIGURE 1.—A diagramatic representation of the proximal *2L* salivary chromosome region. The location of the histone genes and the extent of the deficiencies are indicated by heavy bars.

either a Stubble (*Sb*) or wild-type (Sb^+) phenotype. (2) The length of the posterior sternopleural and posterior dorsocentral macrochaetes was measured using a dissecting microscope equipped with an ocular micrometer. The values obtained from each fly were summed to a single value, which was expressed as a proportion of the difference in length between the fully attenuated bristles of a *Sb* fly and the wild-type bristles of a Sb^+ fly.

Statistics: Analysis of the data obtained from the quantification of variegated phenotypes revealed that the variance of a parameter is often proportional to its mean. The use of Student's *t*-test for the estimation of significance in difference between means is usually restricted to the comparison of groups whose variances are not significantly different. The modification of SUTTERTHWAITE (1946) and WELCH (1951), which retains the method of calculating *t* value, but substitutes an altered estimate of the degrees of freedom, was employed to circumvent this problem.

RESULTS

Definitive evidence that reduction in the multiplicity of histone genes suppresses position effect variegation: MOORE *et al.* (1979) demonstrated that the expression of variegating genes associated with the chromosomal rearrangements Inversion (*1*) white-mottled[4] or Bar of Stone-Variegated-*Y* is increased in individuals that are hemizygous for the 39D-E histone gene complex. That report avoided problems inherent in previous research by KHESIN and LEIBOVITCH (1978), who had employed deficiencies created from *Y*; autosome translocations (such

Y rearrangements have large, unpredictable effects on variegation). There are three criteria for a definitive test of the effect of histone gene deficiencies. First, it is desirable that the deficiencies used are induced on an isogenically derived chromosome. This would reduce potential variation caused by existing variegation-modifying loci (HENIKOFF 1979b). In addition, the deficiencies should be small, so as to minimize possible extraneous effects, such as deletion of intercalary heterochromatin. Third, and more importantly, the control deficiencies should very closely abut the histone gene complex. This would ensure that any difference in variegation modification between the control deficiencies and those deficiencies that remove the histone gene complex would not result from a deletion of genes outside the complex.

To satisfy these criteria, a series of small deficiencies was imposed on an isogenic second chromosome marked with black, purple and cinnabar, using the mutagen triethylene melamine (SINCLAIR, MOORE and GRIGLIATTI 1980). They are labeled *Df(2L)DS*, and their extent is shown in Figure 1. The proximal breakpoint of *Df(2L)DS8* is at the distal edge of the histone gene complex. It is, therefore, a suitable test of the effect of deleting material immediately distal to the complex. A deletion slightly more distal to the complex, *Df(2L)DS9*, provides an additional control. Two deletions, *Df(2L)DS5* and *Df(2L)DS6*, remove the entire 39D-E complex, as well as more distal material [an *in-situ* autoradiograph of *Df(2L)DS5* is shown in Figure 2].

The effect of these four deletions on white$^+$ variegation associated with $In(1)w^{m4}$ was tested. *Df(2L)DS/CyO* males [in which *Df(2L)DS* = *5*, *6*, *8* or *9*] were crossed to $In(1)w^{m4}/In(1)w^{m4}$ females, and the amount of drosopterin pigment in the eyes of w^{m4}/Y; *Df(2L)DS*/+ and w^{m4}/Y; *CyO*/+ F_1 males was quantified. Note that the F_1 progeny receive the deficiency chromosome only from their male parent, eliminating the possibility of a maternal effect on these results. Since these progeny are a first generation outcross from the deficiency chromosome stock, the potential for compensation by histone gene magnification is reduced. The white$^+$ gene functions autonomously in the deposition of pigment in secondary pigment cells. It appears that a variegating gene is either active or inactive in any particular cell (SHOUP 1966); therefore, the relative amount of pigment is representative of the fraction of cells in which the variegating gene is active.

The results enumerated in Table 1 show that *Df(2L)DS8*/+ and *Df(2L)DS9*/+ do not differ significantly from their *CyO*/+ siblings in the extent of w^{m4} variegation. Only *Df(2L)DS5*/+ and *Df(2L)DS6*/+ show markedly increased variegating gene activity. These results confirm previous findings that heterozygous deletions of the 39D-E region suppress position effect variegation (MOORE *et al.* 1979). The results also eliminate the possibility that the difference in variegation modification between the deficiencies is unique to a particular souce chromosome. In addition, since these deficiencies were induced in an isogenic *b*, *pr*, *cn* chromosome, these results also eliminate the possibility that the difference stems from polymorphism within the population from which the deficiencies were derived. Most importantly, these findings reduce the possibility that the observed suppression is due to the deletion of a hypothetical

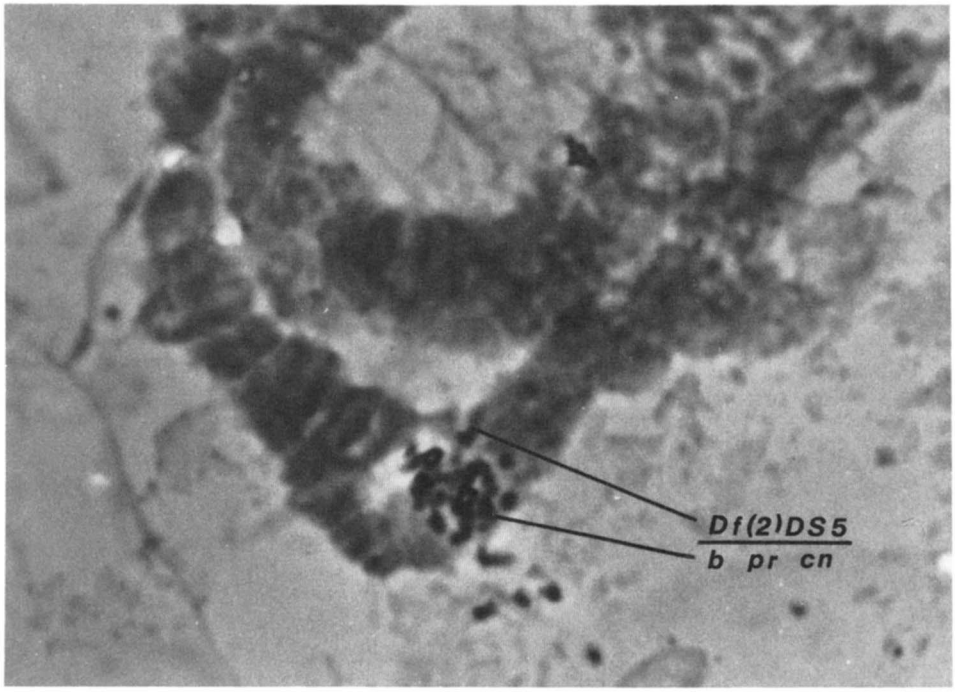

FIGURE 2.—*In-situ* hybridization of ^{125}I-labeled cDm500 plasmid to salivary chromosomes of the genotype *Df(2L)DS5/b pr cn*. Note that the deficiency homolog is devoid of grains.

variegation-modifying locus tightly linked to the distal edge of the histone gene complex.

Activity of a variegating autosomal gene is also increased in the presence of histone gene deletions: Previous studies of the effect of histone gene multiplicity on position effect variegation have focused on two genes, white (3C2) and Bar (16A7), both of which are *X*-linked (KHESIN and LEIBOVITCH 1978; MOORE *et al.* 1979). These loci are subject to dosage compensation, a mechanism that equalizes the overall transcription of most *X*-linked genes in genotypes bearing different numbers of *X* chromosomes. To determine whether the effect of histone gene multiplicity on variegation is restricted to genes that undergo dosage compensation, proximal (*2L*) deficiencies were tested for an effect on variegation of an autosomal gene.

The rearrangement *T(2;3)Sb^V* relocates a mutant allele of Stubble (*Sb*) from its normal position in *3R* euchromatin to the centromeric heterochromatin of *2R*. In this position, the mutant allele, which normally produces an attenuated macrochaete phenotype, displays a variegated expression. Inactivation of Stubble results in the formation of longer bristles, approaching wild-type in phenotype. Conversely, expression of the *Sb* allele produces short macrochaetes.

To test for the effect of histone deletions on *Sb^v*, *CyO/Df(2L)*; +/+ males [in which *Df(2L)* = *Df1*, *12*, *84*, *65* or *161*] were crossed to *SM1,Cy/T(2;3)* Stubble-Variegated (hereafter referred to as *Sb^V*) females and F_1 males and females of the appropriate genotypes were collected and assayed for Stubble variegation

TABLE 1

Mean percentage of the wild-type amount of drosopterin in the eyes of In(1)w^{m4}/Y; Df(2L)/+ *males and their* CyO/+ *siblings*

Genotype	No. of 39DE regions	% white$^+$ drosopterin	$S_{\bar{x}}$	P
w^{m4}/Y;Df(2L)DS8/+	2	12	2.7	>0.05
w^{m4}/Y;CyO/+	2	10	1.8	
w^{m4}/Y;Df(2L)DS9/+	2	6	0.4	>0.05
w^{m4}/Y;CyO/+	2	7	0.4	
w^{m4}/Y;Df(2L)DS5/+	1	25	4.2	<0.05
w^{m4}/Y;CyO/+	2	5	0.6	
w^{m4}/Y;Df(2L)DS6/+	1	38	6.6	<0.05
w^{m4}/Y;CyO/+	2	8	3.6	

n = 30.

according to method 2 (see MATERIALS AND METHODS). The response of Sb^V gene activity to differences in histone gene multiplicity is shown in Table 2. The genotypes bearing the control deficiencies *1* and *12*, which delete regions distal to the histone gene complex, exhibited a low level of Sb^V activity, since their average machrochaete size approached that of wild-type. (A fully wild-type fly has a size value of 100; a fully Stubble fly has a value of O.) The activity of Sb^V was significantly higher (reflected by a shorter average macrochaete length) in those genotypes that delete the histone gene region [*Df(2L)65, 161*]. These results do not preclude the possibility of deficiency effects on the dosage compensation mechanism. They do, however, generalize the effect of heterozygous deletions of the histone gene region to the variegation of autosomal genes. Therefore, we conclude that *X* chromosome dosage compensation is not required for the manifestation of this phenomenon.

A partial deletion of the histone gene complex can cause suppression of position effect variegation: Df(2L)84 lacks both of the darkly staining 39D2 and 3 bands and an indeterminate proportion of the amorphous 39D4-5 region (see Figure 1). The prominent 39E1-2 doublet is intact in this rearrangement. *In situ* hybridization of ^{125}I-labeled cDm 500 plasmid (which carries 1.8 Drosophila histone gene repeats, see LIFTON *et al.* 1977) to squashes of salivary gland chromosomes of the genotype *Df(2L)84/b pr cn* confirms the cytological estimation of this rearrangement as a partial deficiency of the histone gene complement. Grain counts from preparations in which the homologs were clearly separated indicate that the deficiency chromosome possesses approximately 60% of the amount of histone gene DNA present on the normal homolog (G. D. MOORE, unpublished results).

A glance at Table 2 reveals that flies bearing both Sb^V and *Df(2L)84* exhibit increased activity of the Sb^V gene. This effect was equivalent to the suppression of variegation elicited by the total histone deletions *Df(2L)65* or *Df(2L)161*. The effect of various proximal *2L* deletions on *w^{m4}* was tested further by crossing *w^{m4}/Y;CyO/Df(2L)* males [in which *Df(2L) = 1, 12, 84, 65, 161*] to *w^{m4}/w^{m4}*; +/+ females and determining the amount of drosopterin pigment in the eyes of F_1 *Df(2L)/+* and *CyO/+* male and female progeny. The results of this

TABLE 2

Effect of proximal (2L) *deficiencies on bristle length variegation associated with* T(2;3)Sb^V

Genotype	No. of 39DE regions	% of parental Sb^+ bristle length (S_z)	
		Males	Females
T(2;3)Sb^V/Df(2L)1	2	77 (3)	97 (4)
T(2;3)Sb^V/Df(2L)12	2	85 (4)	90 (5)
T(2;3)Sb^V/Df(2L)84	1–2	64 (3)	62 (4)
T(2;3)Sb^V/Df(2L)65	1	71 (4)	74 (3)
T(2;3)Sb^V/Df(2L)161	1	49 (3)	53 (5)

Values were tested for their significance of difference from the average control value for each sex. $P < 0.05$ for each experimental value. $n = 45$.

experiment are shown in Table 3. The percentage of eye cells in which the w^+ gene is expressed in progeny bearing the control deficiencies *Df(2L)1* or *12*, the partial deficiency *84* and the complete histone gene deficiencies *65* and *161* is presented. The suppression of w^{m4} variegation elicited by *Df(2L)84* is within the range of the suppression associated with deficiencies *65* and *161*. Taken together, these results suggest that deletion of the distal portion of the histone gene region is sufficient to elicit a suppression of position effect variegation comparable to that induced by deletion of the entire region. These results also demonstrate that deletion of loci proximal to the histone gene complex is not required for suppression. Since deficiencies that delete only 39E1-2 are not presently available, it was impossible to test whether deletions limited to the proximal portion of the histone gene region can also suppress position effect variegation.

Suppression of variegation evoked by histone deletions does not require the presence of Y *heterochromatin:* Since the *Y* chromosome is known to influence position effect variegation, it seemed possible that the suppressing effect of histone deletions might require the presence of this chromosome. However, a further examination of the effect of histone deletions on the Sb^V (Table 2) and w^{m4} (Table 3) genes suggests that this is not the case. Thus, note that the F_1 female progeny bearing histone deletions exhibit an increase in Sb^V activity comparable to that exhibited by their brothers. The effects of histone deletions on w^{m4} expression in females closely parallel those obtained from the comparable experiment involving w^{m4}/Y males; the proportion of pigment cells exhibiting w^+ activity is greater in those genotypes bearing histone gene deletions [*i.e.*, *Df(2L)65*, *Df(2L)84* and *Df(2L)161*]. Again, the level of pigmentation observed in control deletions which do not affect the histone cluster [*i.e.*, *Df(2L)1* and *Df(2L)12*] is equivalent to that of the nondeficiency-bearing *CyO*/+ females. Therefore, we conclude that the presence of the *Y* chromosome is not required to evoke the modification of position effect variegation associated with histone gene deletions.

A proximal 2L *duplication does not modify position effect variegation:* A possible corollary of the observed suppression of variegation caused by histone gene

TABLE 3

Mean percentage of the wild-type amount of drosopterin in eyes of w^{m4}/Y; Df(2L)/+ *males and* w^{m4}/w^{m4}; Df(2L)/+ *females and their respective* CyO/+ *siblings*

Autosomal genotype	No. of 39DE regions	Males		Females	
		% white$^+$ drosopterin (S_x)	P	% white$^+$ drosopterin (S_x)	P
Df(2L)1/+	2	4 (1.2)	>0.05	3 (0.8)	>0.05
CyO/+	2	3 (0.7)		4 (0.6)	
Df(2L)12/+	2	4 (0.9)	>0.05	5 (1.0)	<0.05
CyO/+	2	3 (0.5)		2 (0.4)	
Df(2L)84/+	1–2	19 (2.4)	≪0.05	24 (4.4)	≪0.05
CyO/+	2	6 (0.7)		5 (0.8)	
Df(2L)65/+	1	9 (2.1)	<0.05	28 (3.5)	≪0.05
CyO/+	2	3 (0.4)		4 (0.9)	
Df(2L)161/+	1	24 (3.2)	≪0.05	12 (5.3)	<0.05
CyO/+	2	8 (1.7)		2 (0.4)	
+/+	2	4 (0.9)		3 (0.5)	

This experiment was done at 17°. $n \geq 40$.

deletions is that a corresponding duplication could enhance variegation. To test this it was necessary to obtain an appropriate duplication and confirm that the duplicated segment contained a substantial portion of the histone complex. *Dp(2;1)C239* (see Figure 3) inserts all, or part, of the 39D-E region within a euchromatic segment of the *X* chromosome. *In situ* hybridization of ^{125}I-labeled DNA from the recombinant plasmid cDM500 to salivary gland preparations from *Dp(2;1)C239* female larvae (the duplication is homozygous and hemizygous lethal) was performed and a representative autoradiograph is presented in Figure 3C. Note the intensity of histone DNA hybridization to the inserted segment, suggesting that a majority of the histone complex is included in this duplication. The duplicated segment is functional, since it complements homozygous deletions of the histone gene complex [*i.e.*, *Dp(2;1)C239*/+;*Df(2L)DS6*/*Df(2L)DS6* is viable]. The next step was to examine the effects of the duplication on position effect variegation, and this was done in two separate experiments. In the first, the effects of *Dp(2;1)C239* on w^{m4} expression were tested by crossing (in three separate matings) w^{m4}/Y;+/+ males to (1) *Dp(2;1)C239w*/*In(1)sc*^{S1}*+d149*; +/+, (2) *w*/*w*; +/+ or (3) *w cv v f*/*w cv v f*; +/+ females, and drosopterin levels were monitored in appropriate F_1 female progeny. The results of this experiment are summarized in Table 4 and show that the levels of pigment deposition are equivalent in both duplication- and nonduplication-bearing females. In the second experiment, the effects of *Dp(2;1)C239* on Sb^V expression were tested as follows. *v*/*Y*;*SM1,Cy*/*T(2;3) Sb*^{V} males were crossed (in two separate matings) to either (1) *Dp(2;1)C239*/*In(1)sc*^{S1}*+d149*; *SM5,Cy*/*In(2LR)bw*^{V} or (2) *dor*^{174}/*In(1)sc*^{S1}*+d149*; *SM5,Cy*/*In(2LR)bw*^{V} females, and appropriate F_1 progeny were examined for Sb^V expression (method 1, see MATERIALS AND METHODS). The results of this experiment are summarized in Table 5. The histone duplication has no effect on the proportion of macrochaete-form-

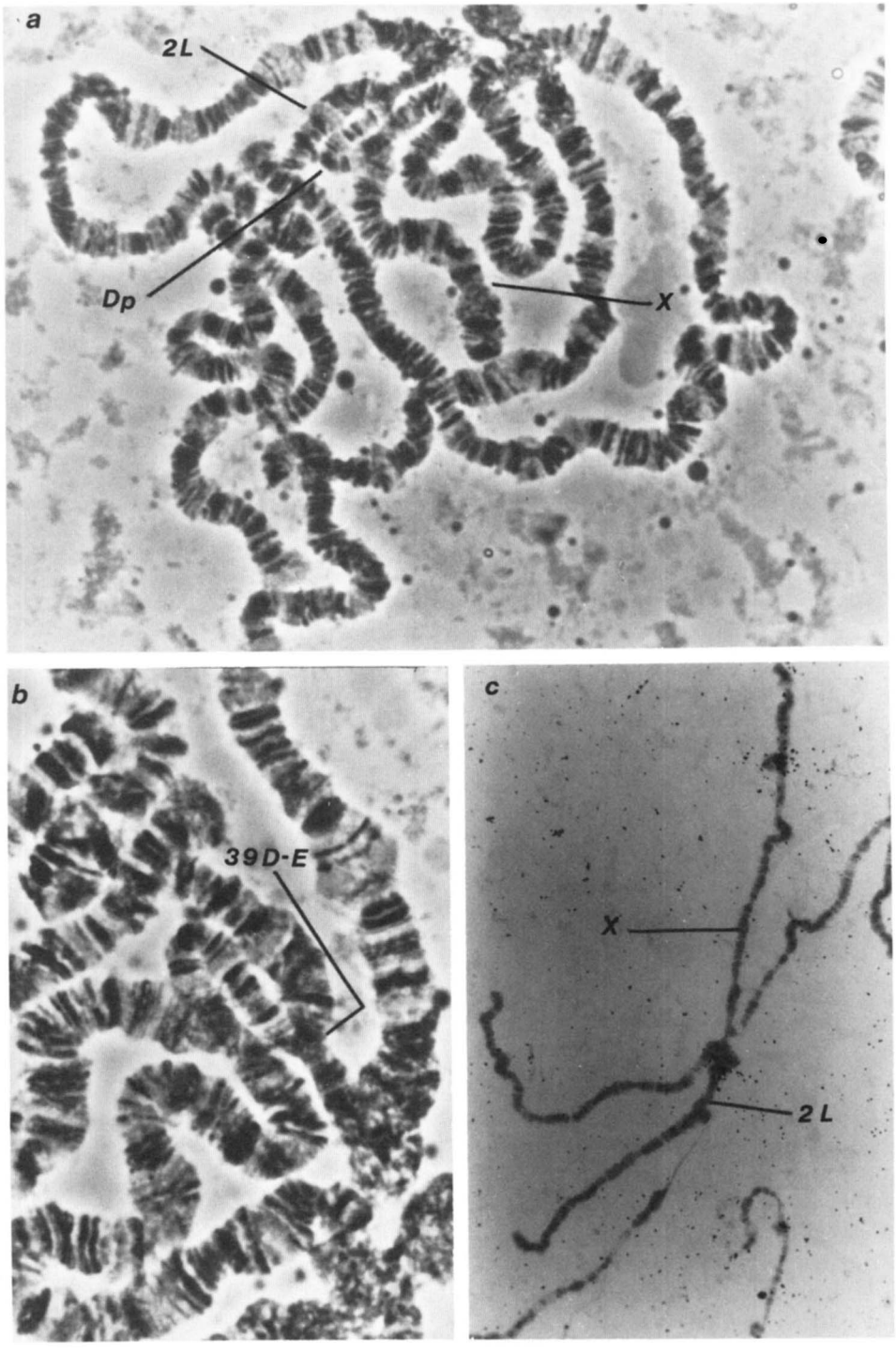

FIGURE 3.—a, Salivary chromosomes of the genotype *Dp(2;1)C239w/w*. b, A magnified view of a. The duplication loop is synapsed to proximal *2L* in the 39D-E region. c, *In situ* hybridization of ^{125}I-labeled cDm500 plasmid to *Dp(2;1)C239w/w* salivary chromosomes. Note the intensity of hybridization to the duplicated segment.

TABLE 4

Mean percentage of the wild-type amount of drosopterin in the eyes of w^{m4} *females*

Genotype	No. of 39D-E regions	% of white$^+$ drosopterin	$S_{\bar{x}}$
Dp(2;1)C239w/w^{m4}	3	7	1.6
w cv v f/w^{m4}	2	7	0.8
w/w^{m4}	2	9	0.8

$n = 25$.

TABLE 5

Mean percentage of dorsocentral and scutellar bristles with a Sb *phenotype in* T(2;3)Sb^v *progeny from proximal* (2L) *duplication and nonduplication mothers*

Progeny	No. of 39D-E regions	No. of maternal 39D-E regions	% *Sb* bristles	$S_{\bar{x}}$
Cross (1)				
DpC239/v;Sb^V*/Cy*	3	3	47	14
DpC239/v;Sb^V*/bw*^{V}	3	3	52	9
dl49/v;Sb^V*/Cy*	2	3	55	9
dl49/v;Sb^V*/bw*^{V}	2	3	44	6
dl49/Y;Sb^V*/Cy**	2	3	49	11
dl49/Y;Sb^V*/bw*^{V}*	2	3	67	9
Cross (2)				
dor^{174}*/v;Sb*^{V}*/Cy*	2	2	45	10
dor^{174}*/v;Sb*^{V}*/bw*^{V}	2	2	46	6
dl49/v;Sb^V*/Cy*	2	2	48	5
dl49/v;Sb^V*/bw*^{V}	2	2	46	7
dl49/Y;Sb^V*/Cy**	2	2	45	10
dl49/Y;Sb^V*/bw*^{V}*	2	2	69	6

* Due to the sc^{S1} phenotype, only the dorsocentral bristles could be scored. $n \geq 25$.

ing cells exhibiting inactivation of Sb^V (*e.g.*, compare lines 1 with 3 and 2 with 4). These results are identical with those obtained in the previous experiment with w^{m4}. Taken together they clearly suggest that increasing the number of 39D-E segments by approximately 50% elicits no modification of position effect variegation.

The histone gene dosage of the maternal parent does not influence position effect variegation in her euploid offspring: In Drosophila embryos, transcription commences at the syncytial blastoderm stage, approximately 90 min after oviposition (ZALOKAR 1976). Maternal histone mRNA, which constitutes 2% of the mRNA in the preblastoderm embryo, is responsible for the formation of histones prior to this time (ANDERSON and LENGYEL 1980). HARTMANN-GOLDSTEIN (1967) determined that the temperature-sensitive period for white variegation occurs during the first 4 hr of embryogenesis, a period that encompasses the first appearance of heterochromatin (MAHOWALD 1968). It is conceivable that alteration of maternal histone gene multiplicity could affect the accumulation of maternal histone mRNA in the oocyte. In turn, this could

alter the amount of cellular histone available during the period in embryogenesis when gene inactivation due to position effect variegation occurs.

To examine the effects of maternally borne histone gene deletions on position effect variegation in euploid offspring, the following experiment was performed. w^{m4}/Y; +/+ males were crossed to w^{m4}/w^{m4};*CyO/Df(2L)* females [*Df(2L)* = *Df(2L)1*, *12*, *84*, *65* or *161*], and the levels of drosopterin deposition were monitored in appropriate F_1 progeny. The results of this experiment are presented in Table 6. Note again that the maternal genotypes of *Df 1* and *12* serve as the controls as compared with the *Df 84*, *65* and *161* (histone gene deletion) genotypes. A glance at the results shows no significant differences in pigment levels between *CyO*/+ progeny (males or females) from histone deficiency or control mothers, indicating that heterozygous deletions of the histone region exert no apparent maternal affect on w^{m4} variegation. In addition to this finding, any maternal effect associated with a duplication of 39D-E would be evident from the Sb^V experiment involving *Dp(2;1)C239* (see Table 5), since the $In(1)sc^{S1}+d149/v$ and $In(1)sc^{S1}+d149/Y$ progeny were obtained from either duplication- or nonduplication-bearing mothers. The results show that, in fact, the variegated phenotypes of these progeny were not significantly altered by differences in maternal histone gene multiplicity (*e.g.*, compare progeny in lines 3 and 4 with those in lines 9 and 10, respectively). Hence, we can conclude that alterations in histone gene multiplicity (either increases or decreases) do not elicit a significant maternal effect with regard to position effect variegation. There are two obvious alternative interpretations of this result: (1) Alteration of the histone gene complement in the maternal genotype is not rate determining on the accumulation of histone mRNA in the oocyte. (2) The transcriptional fate of the variegating gene is determined after the time at which histones coded by the embryo supercede histones translated from maternal mRNA.

DISCUSSION

ZUCKERKANDL (1974), in a review of the structure of heterochromatin, suggested that the "spreading effect" of gene inactivation characteristic of position

TABLE 6

Mean percentage of the wild-type amount of drosopterin in the eyes of w^{m4}/Y; CyO/+ *and* w^{m4}/w^{m4}; CyO/+ *progeny from* CyO/Df(2L) *mothers*

Maternal genotype	No. of maternal 39D-E regions	% w^+ drosopterin (S_x)	
		Males	Females
CyO/Df(2L)1	2	6 (0.6)	2 (0.2)
CyO/Df(2L)12	2	3 (0.4)	1 (0.2)
CyO/Df(2L)84	1–2	4 (0.7)	2 (0.3)
CyO/Df(2L)65	1	3 (0.4)	1 (0.2)
CyO/Df(2L)161	1	3 (0.5)	1 (0.1)

Values were tested for their significance of difference from the average control value for each sex. $P > 0.05$ for each of the experimental values. $n = 20$.

effect variegation was the result of the diffusion of "locking molecules," which confer the cytochemical properties of heterochromatin, into chromosomal regions adjacent to the heterochromatic breakpoint. His proposal that histones might act as agents of the spreading effect is supported by the observation that sodium butyrate, which reduces the affinity of histone for DNA by blocking deacetylation, reduces the gene inactivation associated with the position effect variegation of w^{m4} (MOTTUS, REEVES and GRIGLIATTI 1980). The present study provides definitive evidence that large reductions in the number of histone genes suppress the inactivation of variegating genes. None of the five deficiency strains which cumulatively delete euchromatic segments from the distal edge of the histone gene cluster (39D2) to 37F has any effect on variegation. In contrast all of the five deletions that remove a substantial portion of the cluster, or the entire cluster, cause marked suppression of variegation. This is observed, regardless of the genetic constitution of the chromosome bearing the deletion. Hence, there appears to be a definite correlation between reduction in the number of histone templates and suppression of position effect variegation.

It should be mentioned that KHESIN and LEIBOVITCH (1978) had advanced a similar argument, based on their finding that a deletion constructed from T(Y;2) stocks with different autosomal breakpoints in proximal *2L* caused suppression of $T(1;3)w^{vro}$ variegation. Since initial cytological examination had indicated that this synthetic deficiency encompassed the 38B-40 region (which would include the histone genes), the authors suggested that the suppression effect was due to a deletion of the histone gene complex. However, their study is subject to two major criticisms. First, it has been established that T(Y;2) rearrangements, as well as deletions and duplications constructed from them, frequently have very strong and unpredictable effects on variegation (ranging from enhancement to suppression) depending upon the *Y* chromosome breakpoints involved (MOORE 1980). Therefore, it is difficult to obtain adequate controls for experiments involving these rearrangements. Second, recent cytological analysis has suggested that the autosomal breakpoint of the proximal T(Y;2) element used by KHESIN and LEIBOVITCH (*i.e.*, B190) is actually in 39C, rather than 40 (see SIEGEL 1981). If this is true, then the deletion used by the former workers would not affect the histone cluster. For these reasons it is difficult to draw any firm conclusions about the effect of histone deletions on position effect variegation from this earlier study.

Our results demonstrate that heterozygosity for deletions of the 39DE region causes a suppression of variegation. There are two simple conclusions that can be reached from these data. Either a reduction in the number of histone templates suppresses the inactivation of variegating loci or a locus exists in the region of the histone gene complex which, when deleted (or presumably when mutated), is capable of suppressing position effect variegation (MOORE *et al.* 1979). We favor the former hypothesis, although the latter cannot be disproven unequivocally. Indeed, SPOFFORD (1976) has identified several loci that can suppress variegation. On the basis of his analysis of a small region in the right arm of chromosome *3*, HENIKOFF (1979b) suggested that loci that are capable of modifying variegation may exist at a frequency as high as 1 per 25

chromomeres. If such hypothetical loci exist within the 39DE region, our studies suggest that they must reside within the histone gene complex. No dose-sensitive locus capable of suppressing variegated position effects exists within the region deleted by *Df(2L)12*, which is comprised of at least 60 bands, 38A through 39C. More importantly, *Df(2L)DS8*, which deletes chromosomal material up to, and probably including, the distal terminus of the histone gene complex, does not suppress variegation, whereas *Df(2L)84* does suppress position effect variegation. Therefore, if such suppressor loci exist in the 39DE region, they must be located within the distal portion of the histone gene complex itself. Hence, the agents of suppression are either the histone genes themselves or nonhistone genes which map within the cluster. The latter possibility is unlikely for the following reasons. First, of more than 50 EMS-induced dominant suppressors of variegation isolated and analyzed in our laboratory, none maps to the 39DE region (R. MOTTUS, unpublished results). Second, we have strong evidence that few, if any, single copy genes are located within the histone gene complex. Of 140 EMS-induced recessive lethals obtained which map within the 24 band region deleted by *Df(2L)DS6*, few map within the histone gene complex (SINCLAIR *et al.* 1982; G. D. MOORE, D. A. SINCLAIR and T. A. GRIGLIATTI, unpublished results). Although the possibility that redundant variegation-suppressing genes exist within the histone gene complex has not been eliminated, we feel that it is more reasonable to suggest that suppression is caused by deletions of the histone templates *per se*.

Our results demonstrate that hemizygosity for the histone gene complex suppresses variegated position effects exerted by *Y* heterochromatin (B^{SV}; BROSSEAU 1960) and the heterochromatin of autosomal and *X* chromosome centromeres (Sb^{V}, w^{m4}). Dosage compensation mechanisms, or the suppressive action of the *Y* chromosome, are not essential components of modification associated with reduction in histone gene multiplicity. The generality of the histone gene multiplicity effect is consistent with a model of position effect variegation in which diffusion of heterochromatic histone results in the spreading effect. Consonant with this model is the notion that the *Y* chromosome exerts a modifying effect by acting as an alternate target for these molecules. Although it satisfies the current data, this model may be somewhat simplistic. As an alternative, one can speculate that a reduction in the amount of histone, or its affinity for DNA, merely has an antagonistic effect to the gene-inactivating properties of whatever heterochromatic component is responsible for propagating the spreading effect.

Although it is clear that deficiencies of the 39DE histone gene region suppress position effect variegation, the molecular implications of this observation are obscure. For example, a direct effect of these deficiencies on the level of cellular histone has not yet been demonstrated. A reduction in histone protein equivalent to the 50% reduction in 39DE segments in deficiency heterozygotes seems improbable. Indeed, CHERNYSHEV *et al.* (1980) and CHERNYSHEV (1982) have reported partial compensation for a deficiency of proximal *2L* by magnification of the histone genes on the nondeficiency homolog. Preliminary results of saturation-hybridization experiments conducted in our laboratory

confirm their findings of histone gene multiplicity greater than 50% in stocks heterozygous for deficiencies of the 39D-E region (G. D. MOORE, unpublished results). However, amplification of histone genes among F_1 outcrossed individuals heterozygous for a histone deletion is low, generally about 10–15%. Modification of variegation in deficiency genotypes suggests that any such compensation does not fully restore a wild-type level of histone protein.

Interestingly, whereas reductions in histone gene multiplicity suppressed variegation, an increase in the number of histone genes had no apparent effect on variegation (Tables 4 and 5). This suggests that histone protein production has an upper limit, not dependent on gene multiplicity, or that chromatin structure is unaffected by a super abundance of histones. The latter explanation is unlikely, given the findings of SPERLING and WEISS (1980), who have demonstrated that chromatin with a characteristic internucleosomal spacing will alter its spacing in response to cell fusion with a cell type having a different spacing length. NELSON, HSIEH and BRUTLAG (1979) have isolated a factor from Drosophila embryos that mediates the assembly of nucleosomes on DNA. It is possible that the abundance of this factor limits the rate at which histone is incorporated into chromatin. Interestingly, OSLEY and HEREFORD (1981) demonstrated a dose-dependent effect on transcription resulting from the transformation of a yeast-derived DNA fragment containing the H2A and H2B genes and an adjacent nonhistone gene, into the yeast genome. Compensation for the increased transcriptional capacity is accomplished by an increased rate of histone mRNA degradation. This form of compensation did not extend to the mRNA transcribed from the adjacent duplicated nonhistone gene, implying the existence of a mechanism which specifically regulates the size of the histone mRNA pool. The observation that a duplication of the Drosophila histone gene region has no effect on the expression of genes subject to position effect variegation could be interpreted to suggest that such a compensatory mechanism also exists in this organism.

The observation that *Df(2L)84*, which deletes only the distal 40–50% of the histone gene complex, causes a level of suppression comparable to that of deletions that completely remove the histone gene complex is extremely intriguing. Such a finding might indicate that suppression is quantitative with regard to histone deletions; however, once a threshold is reached in terms of loss of histone templates, the maximum suppression is elicited. An alternative, and more attractive, explanation of this finding is that the histone gene complex is organized in a nonhomogeneous fashion and that the organization reflects differential expression of particular subsets of repeated genes during different stages of development. The latter is particularly intriguing in light of recent studies of the molecular organization of the histone repeat units. LIFTON *et al.* (1977), KARP (1979) and GOLDBERG (1979) have determined that the majority of the repeated units comprising the histone gene complex are of two forms, either 4.8 or 5.0 kb, both of which encode all five major histone mRNAs. The 5-kb form outnumbers the 4.8-kb form by a 3:1 ratio. Although large fragments containing adjacent 4.8- and 5-kb repeats have been generated, the two principal variants are not randomly interspersed, since fragments con-

taining a 4.8-kb repeat sandwiched between 5-kb repeats cannot be detected. On the other hand, restriction analysis of the histone gene regions of Drosophila embryos and cultured cells (SAIGO, MILLSTEIN and THOMAS 1980) has demonstrated that the tandemly reiterated structure of repeats is not uniform. The existence and nonrandom distribution of these distinct repeat units, coupled with the potential for developmental heterogeneity with regard to histone subtypes (*e.g.*, D2; PALMER, SNYDER and BLUMENFELD 1980) suggest that the histone complex should be amenable to genetic dissection. Indeed, from complementation analysis of mutations in proximal (*2L*), SIEGEL (1981) concluded that the 39D2-3 to E1-2 region, which encompasses most of the histone genes, can be subdivided into three lethal complementation groups. Homozygous deletions of subsets of the histone gene repeats, even though they presumably reduce total histone gene multiplicity by less than 50%, cause lethality. This finding may indicate that complete deletion of certain subsets of the histone gene repeat sequences, or other genes in the 39DE region, prevents the occurrence of specialized vital functions.

In addition to modification of position effect variegation, other stage-specific genetic and biochemical phenomena may be influenced by lesions in the histone gene function. These include recombination frequency, and mutagen and nuclease sensitivity. These studies, as well as others that seek to determine the relationship between histone gene numbers and the histone mRNA pool size, are currently being undertaken in our laboratory.

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