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

by

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ABSTRACT

The effect of altered histone gene multiplicity on chromatin structure was assayed by measuring changes in gene activity associated with position-effect variegation. Heterozygous deficiencies of the histone gene cluster of *Drosophila melanogaster* increased the proportion of cells within a tissue in which a variegating gene was active. This effect was not dependant on the Y chromosome and applied to both X-linked and autosomal variegating genes. Deletions of the histone gene complex imposed on different source chromosomes elicited the same response. Partial deficiencies, which delete different regions of the cluster, varied in their effect on variegation. Duplications of the histone gene cluster did not increase the proportion of cells in which a variegating gene was inactive. The presence of deficiencies or a duplication of the cluster in the maternal genome did not modify the extent of position-effect variegation in their progeny. These results are discussed with respect to current knowledge of the organization of the histone gene cluster and control of its expression.

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INTRODUCTION

The five histone proteins are small, basic polypeptides whose responsibility for the packaging of DNA into the primary unit of chromatin, the nucleosome, has been extensively documented (27). 146 base pairs of DNA are coiled around the exterior of a core particle composed of two molecules each of histones H2A, H2B, H3 and H4 (18). A single molecule of histone H1 is complexed with a more variable length of DNA (usually about 60 base pairs) which links adjacent nucleosomes (43). The action of H1 is thought to confer higher order structure on chromatin (13). The amino acid sequence of each of the five histone proteins is highly conserved among the majority of eukaryotes. This suggests that each region of these molecules has an invariant interactive function. Ready availability of nearly pure preparations of histone messenger RNA from sea urchin embryos, which could be directed as a hybridization probe against the histone genes of diverse species, allowed these genes to be among the first accessible to biochemical analysis (31,32). In all species which have been examined (with the exception of the yeast *Saccharomyces cerevisae*) there is evidence that the coding sequences of the five principle histone proteins are grouped together, separated by relatively short lengths of non-coding DNA (33). This unit is tandemly reiterated to a moderate repetitivity which varies between species (Table I). In *Drosophila* and humans,

TABLE I. Histone Gene Reiteration in Various Species

Species	Number of Repeat Units per Haploid Genome	Reference
Drosophila	110	(36)
Man	40	(54)
Chicken	10	(14)
Xenopus	20 - 50	(28)
Yeast	1*	(33)
Sea urchin	200 - 500	(31)

* H2A and H2B only.

the repeat units are clustered in a single chromosomal segment (12,44). The reason for this redundancy has not been elucidated. It has been suggested that it is necessary to meet the demand exerted on the transcriptional capacity of these genes during periods of rapid chromosome replication (28).

Several studies have been attempted to correlate changes in the activity of histone genes to events in the cell and developmental cycles (for a review see BORUN, (6)). Mutational analyses, which assess alterations in histone gene products or chromatin structure resulting from specific changes in histone gene structure or multiplicity, have been rare. Few organisms possess sufficiently well established genetics to recover such mutations (although the new "psuedo-genetics" which uses cloned and specifically restricted DNA in oocyte or cell-free systems of transcription and translation, is rapidly sidestepping this problem). The histone gene unit of *Drosophila melanogaster* is reiterated about 110 times per haploid genome (36). These repeated units are clustered in the proximal left arm of the second chromosome in salivary gland chromosome bands 39D2-3 to E1-2 (44). Hence, they are accessible to genetic manipulation. Duplication of, or deficiency for, one entire cluster does not result in lethality, nor does it have an overt phenotypic effect. The aim of this investigation is to examine the effect of altered histone gene multiplicity on chromatin morphology and gene expression by monitoring a genetic phenomenon, position-effect variegation, which is sensitive to changes in chromatin structure.

Position-effect variegation was originally observed in *Drosophila* by MULLER (40) and has since been demonstrated in plants and mammals (11,2). When cells bear a chromosomal rearrangement which juxtaposes a euchromatic gene to heterochromatin, a fraction of the cells exhibit no expression of that gene. The resultant organism is a mosaic for the activity of the rearranged gene. The degree of mosaicism can be modified; factors such as elevated developmental temperature, additional Y chromosome heterochromatin in the genome, and various modifier genes enhance the proportion of cells in which a variegated gene is active. Although the phenomenon has been extensively catalogued (reviews by BAKER, (2); and SPOFFORD, (49)) the underlying molecular mechanism of position-effect variegation is unclear. Two lines of evidence suggest the variable genetic activity resulting from the position-effect is related to altered chromatin morphology at the variegating locus:

i) If two genes are involved in a rearrangement, the gene nearest to the heterochromatic break point will be inactivated in a greater proportion of cells (16).

ii) For gene loci which can be assigned to specific salivary gland chromomeres (bands), the intensity of gene inactivation following rearrangement is proportional to the fraction on salivary cells in which the locus has assumed a heterochromatic morphology (25).

It has been proposed that the variegating gene locus is condensed and transcriptionally inactivated by a "spreading

effect" or limited spatial diffusion of molecules from the adjacent heterochromin (59). The nature of heterochromatic elements which could effect this condensation is open to speculation. The cytological and genetic properties which define heterochromatin such as condensed staining throughout the cell cycle, a paucity of genes, and reduced recombination do not illuminate its molecular constitution (56). As opposed to its nucleic acid component, which consists primarily of highly reiterated, very short sequences, there is little information about the distinctive features of heterochromatic proteins (10). BERLOWITZ (3) reported that the heterochromatic chromosome set of the mealybug is enriched in histone content. In the genus *Drosophila*, the types of phosphorylated sub-species of the histone H1 vary between species differing in heterochromatic composition (5). One can hypothesize that heterochromatic histones are agents of the spreading effect. A prediction of this model is that variation in the amount of cellular histone protein should alter the extent of any morphological change in chromatin associated with the position effect. To test this prediction, the effect of altered histone gene multiplicity on the degree of mosaicism associated with variegating genes has been examined.

MATERIALS AND METHODS

1. Culture Conditions and Mutant Stocks

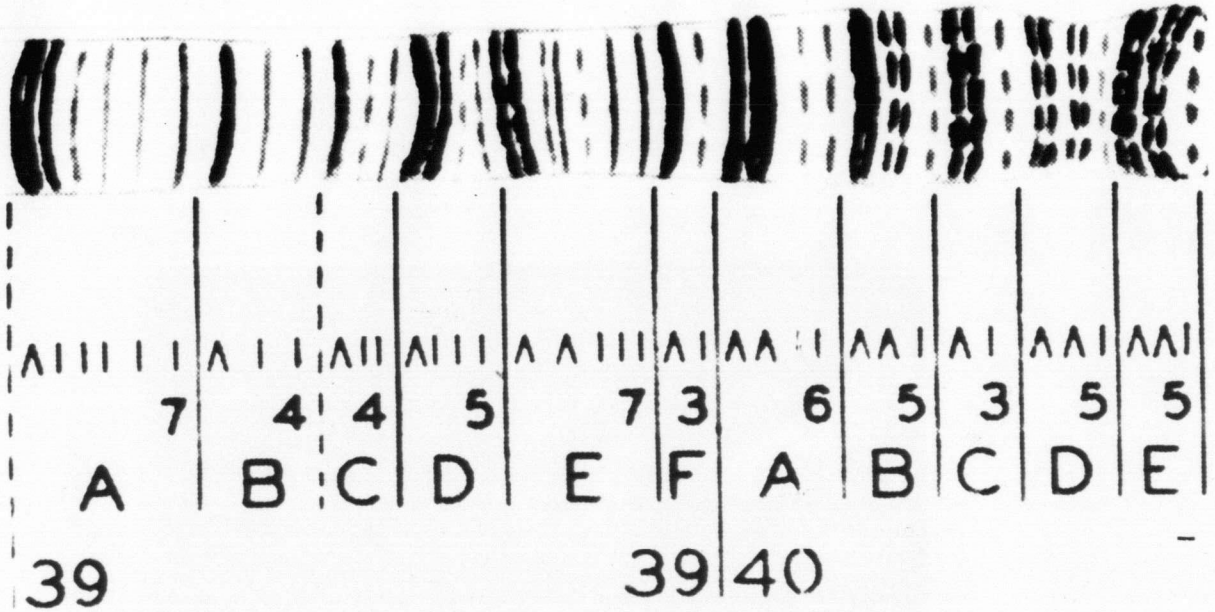
Flies were reared in ¼ pint milk bottles or shell vials on a sucrose - cornmeal - agar medium, seeded with bakers' yeast. Tegosept (methyl - p - hydroxybenzoate) was included in the food as a mould inhibitor. To suppress bacterial growth, a combination of streptomycin and tetracycline or ampicillin and tetracycline was included (10mg/litre, each).

Descriptions of the mutations and chromosomes used can be found in LINDSLEY and GRELL (37), with the following exceptions:

(i) *Bar of Stone-variegated* (B^{SV}). This chromosome is an irradiation-induced derivative of $B^{S}Y$ which exhibits a variegated position-effect of the mutation *Bar*. It is described by BROUSSEAU (8).

(ii) Proximal (2L) deficiencies. *Df(2)1, 12,65,84* and *161* were generated in screens for deficiencies of the dopa decarboxylase locus perfomed by WRIGHT et al. (55). *1,12,65* and *84* are X-irradiation induced derivatives of-a *Tuft lethal (2)74i* chromosome, while *161* was induced by X-rays on a *cinnabar (cn) brown(bw)* chromosome. The original cytological description was reconfirmed and is presented in Figure 1. Their genetic constitution is indicated below:

Df(2)1,12,64,84 are deleted for *purple(pr), Bristle(Bl) and lethal (2)cryptocephal(crc). Df(2)161* is deleted for *pr, Bl,l(2)crc, Minute(2)H(M(2)H).*



Histone

37E2-F4 ←---- Df(2L)12

38A7-B1 ←---- Df(2L)1

37F5-38A1 ←---- Df(2L)84

37F5-38A1 ←------- Df(2L)65

38A6-B1 ←------ Df(2L)161

Figure 1. Cytological map of the proximal (2L) deficiences of WRIGHT et al. (55).

Nine mutations of proximal (2L) were imposed with the mutagen, triethylmelamine, on a *black(b) pr cn* chromosome by SINCLAIR et al. (46). A cytological examination of these mutants *(DS1-9)* revealed that at least five of the mutants are visible deficiencies, as indicated in Figure 2.

(iii) *Translocation(Y;Autosome)* aneuploids. The generation of this series of chromosomes, and their use in creating segmental aneuploids of autosomal loci, is described in LINDSLEY and SANDLER et al. (38). An example of segmental aneuploid synthesis by this technique is illustrated in Figure 3.

(iv) *Duplication(2;1)C239*. This X-linked duplication of proximal (2L) is a segregant from a *Translocation(1;2)C239* stock. The scheme for the creation of a duplication stock is indicated in figure 4. In-situ hybrization of ^{3}H labelled RNA transcribed from recombinant plasmid *cDM500*, which carries the *Drosophila* histone gene unit, revealed that the duplicated segment contains all, or a portion of, the histone gene cluster (Figure 5)(36).

2. Eye Pigment Measurement

In order to limit the scale of the experiments it was desirable to measure the eye pigment content of individual heads. A fluorometric technique was developed to permit measurements having the necessary precision. Flies were allowed to age 1-7 days post-eclosion, then decapitated by freezing and agitation. Single heads were crushed on cellulose chromatography plates (Eastman 13255) and pigments were separated with a 2:1 propanol:1% aqueous NH_3 solution. The drosopterin spot was located and the relative level of fluorescence was measured using a Zeiss micro-

Histone

I(2)DS2

I(2)DS5

I(2)DS6

I(2)DS8

I(2)DS9

Figure 2. Cytological map of the proximal (2L) deficiencies of SINCLAIR et al. (46).

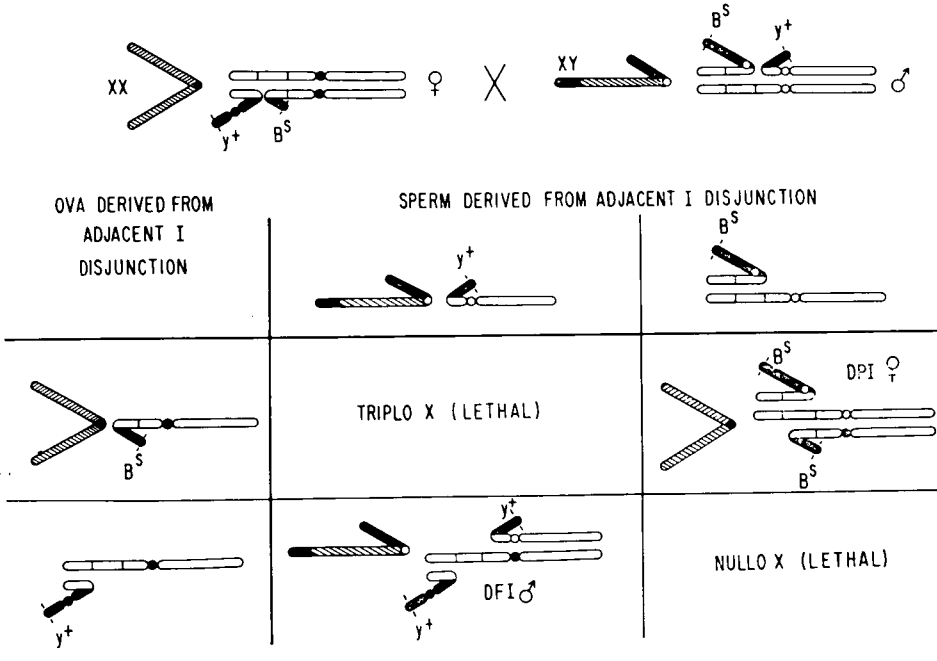
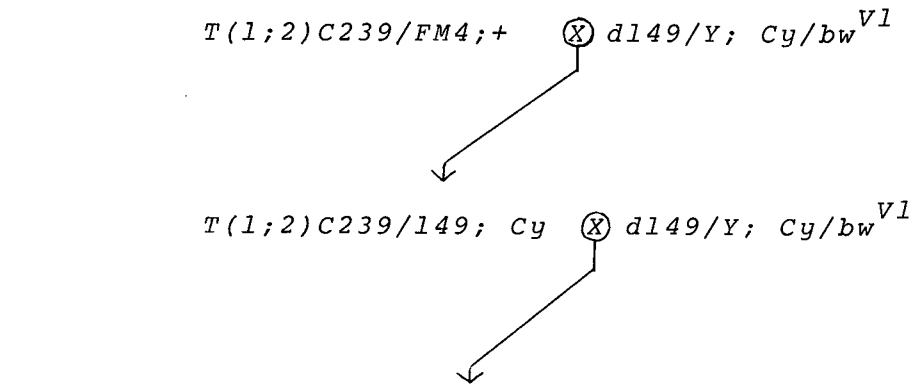

XX
y+
BS
♀
X
XY
BS
y+
♂
OVA DERIVED FROM ADJACENT I DISJUNCTION
SPERM DERIVED FROM ADJACENT I DISJUNCTION
y+
BS
BS
TRIPLO X (LETHAL)
BS
DPI ♀
BS
y+
y+
DFI ♂
NULLO X (LETHAL)

Figure 3. The production of segmental aneuploids from *T(Y;A)* translocations. From LINDSLEY and SANDLER (38).



d149=Inversion(1)scute^{S1} + delta 49, sc^{S1} v f car

FM4 = In(1)FM4, y^{31d} sc^{8} w dm B

Cy = In(2LR)SM5, al Cy lt^{v} cn^{2} sp^{2}

Figure 4. Scheme for the generation of the *Dp(2;1)C239* stock.

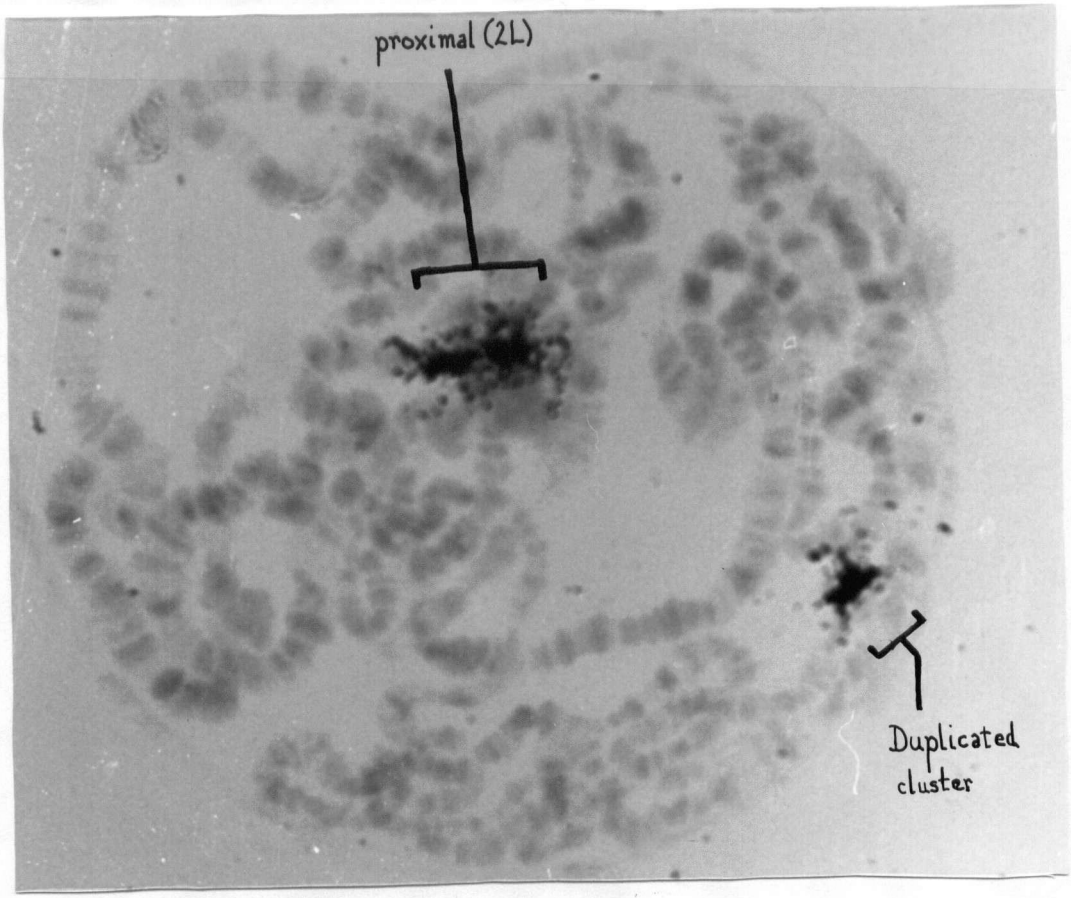

proximal (2L)
Duplicated
cluster

Figure 5. Photomicrograph of a polytene chromosome spread from the *Dp (2;1)C239* stock showing in - situ hybridization of cRNA from plasmid cDm 500.

scope equipped with a UV light source and a photomultiplier. The fluorometer was standardized against the amount of drosopterin fluorescence from *white*$^{+}$ (Oregon-R strain) heads. To ensure that the fluorescent response was linear over the range of pigmentation measured, a drosopterin extract (in ethanol, pH2) dilution series was prepared, chromatographed and measured (17). The response curve is illustrated by Figure 6.

3. Eye Size Measurement

Eye shapes were sketched to scale on gridded bond paper with the aid of a dissecting microscope equipped with an occular micrometer. The outlines were cut out and weighed to estimate surface area relative to wild-type eyes.

4. Bristle Phenotype Measurement

Bristle phenotype was scored by either of two methods, dependant on the genotypes involved in the experiments:

(i) The dorso-central and scutellar bristles were observed by dissecting microscope and assigned a *Sb (Stubble)* or *Sb+* phenotype individually.

(ii) Genotypes bearing *Tft* or *Bl* were difficult to score unambiguously by the previous method since both of these mutations perturb bristle morphology. In these cases the length of the posterior sterno-pleural and posterior dorso-central bristles were measured using a dissecting microscope equipped with an occular micrometer. The values obtained from each fly were summed to a single value from which was substracted the length of the four corresponding bristles in a *Sb* fly. This new value was divided by the difference in

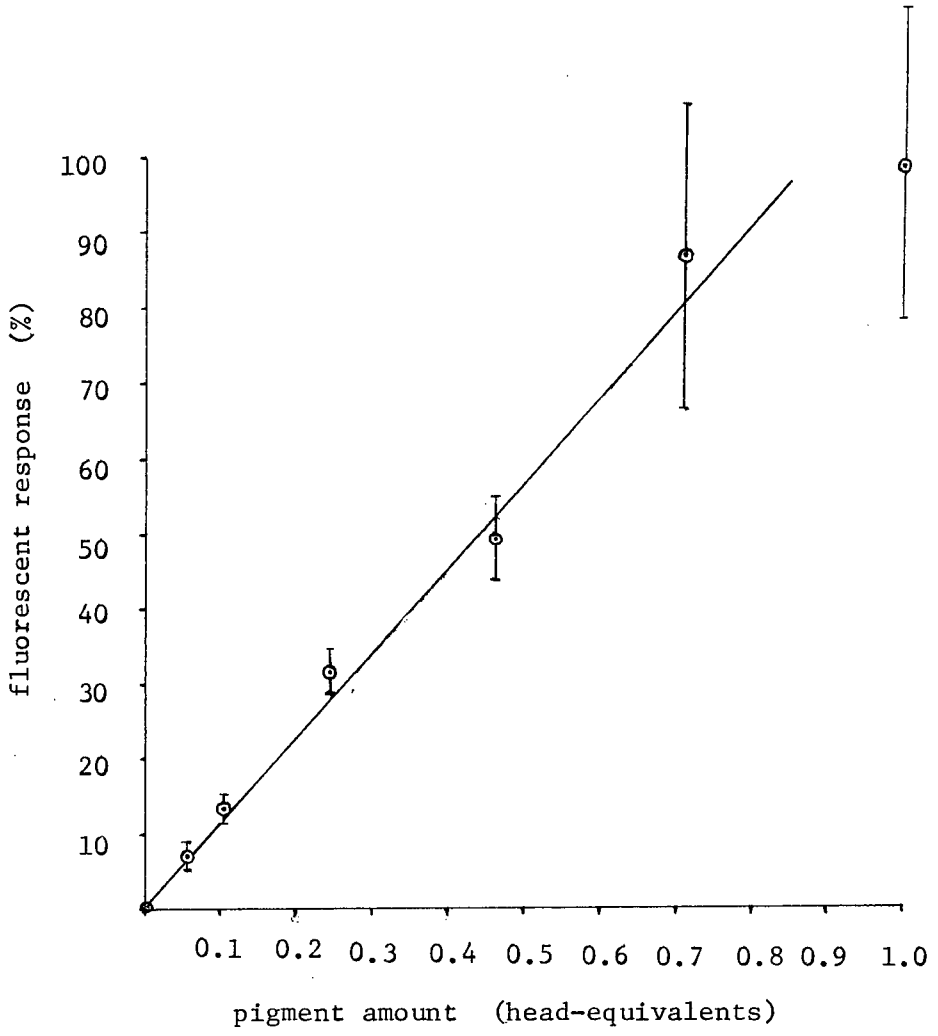

fluorescent response (%)
100
90
80
70
60
50
40
30
20
10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
pigment amount (head-equivalents)

Figure 6. Fluorometric response versus amount of drosopterin pigment.

length between the corresponding sb^+ parental bristles and sb bristles. The formula is illustrated below:

$$\frac{(sb^V \text{ s.-p. and d.-c. bristle length} - sb \text{ bristle length})}{(sb^+ \text{ bristle length} - sb \text{ bristle length})} \times 100\%$$

A variegated bristle phenotype which is more wild-type (sb^+) will have a value approaching 100%. Those phenotypes which are more sb in appearance will have values approaching 0%.

5. Statistics

Analysis of data obtained from quantification of variegated phenotypes revealed that the variance of a parameter is often proportional to its mean. Student's t - test, which estimates the significance of difference in means between groups can only be used to compare groups whose variances are not significantly different. It was necessary to employ a modification of the t - test which accounts for difference in variances between groups in order to analyse the data. The modification of SUTTERTHWAITE (51) and WELCH (53) retains the method of estimating the t value, but substitutes an altered estimate of v (total degrees of freedom) which is $\hat{f}$.

$$\hat{f} = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}{\left[\frac{\left(\frac{s_1^2}{n_1}\right)^2}{f_1} + \frac{\left(\frac{s_2^2}{n_2}\right)^2}{f_2}\right]}$$

$$\hat{f} \leq (f_1 + f_2)$$

RESULTS

1. The Effect of *T(Y;2)* Segmental Aneuplodies of the Histone Gene Cluster on Variegation of w^{m4}.

Stocks bearing segmental deficiencies or duplications can be created from crosses of appropriately marked *T(Y;A)* stocks (Figure 3). The boundaries of the segmental aneuploidy are defined by the translocation break points. KHESIN and LIEBVOTICH (34) exploited the ease and flexibility of this technique to test the effect of a deficiency of the histone gene cluster on the position-effect variegation of the *white* gene associated with $T(1;3)W^{VCO}$, and X-chromosome transcription in intersexes. There is, however, a serious theoretical reservation to the use of Y chromosome rearrangements in the study of position-effect variegation. The Y chromosome contains regions which are potent suppressors of variegation, and their integrity in *T(Y;A)* chromosomes is uncertain (9). Moreover, the *T(Y;A)* aneuploid generation technique produces poorly defined deficiencies and duplications of Y chromosome material. Inherent in its use are difficulties in properly controlling for the effect of the Y chromosome on variegation. In order to determine whether the segmental aneuploidy technique might be of further use in the study of histone gene multiplicity effects on variegation, a series of *T(Y;2)* deficiency, non-deficiency and duplication chromosomes were tested for modification of the variegated phenotype associated with

Inversion(1)white-mottled^{4}. A generalized scheme for these tests is displayed in Figure 7.

The rearrangement, *In(1)*w^{m4}, relocates the *white*$^{+}$ gene from its normal position near the tip of the X chromosome to the centromeric heterochromatin. In its normal chromosomal position, the *white*$^{+}$ gene functions in the formation of pigment granules in secondary pigment cells of the eye (58). Individual cells bearing the inversion genotype exhibit either a full complement of pigment granules or none (45). This results in eyes which have a mosaic pigmentation pattern in *In(1)*w^{m4} flies. In these tests, the extent of the *white*-variegated phenotype was quantified by measuring the relative amount of the eye pigment, drosopterin, in individual heads. The effect of the *T(Y;2)* aneuploidies on the w^{m4} position effect is detailed in Table II. The various segmental aneuploidies of the histone gene cluster and adjacent regions affect the mosaic phenotype markedly, but there is no consistent pattern of suppression or enhancement of variegation with respect to deletion or duplication of the histone gene cluster. *T(Y;2)* translocations which were not aneuploid had a similar pronounced effect on the variegated phenotype. These results suggest that alterations of Y chromosome loci, rather than histone gene multiplicity, is responsible for the observed modification of the variegated

17°

XY/O;Cy/T(Y;2)♂♂ ⊗ w^{m4}/w^{m4}; +/+ ♀♀

↓

w^{m4}/O; +/T(Y;2) ♂♂

17°

+/Y; CyO/Df(2)♂♂ ⊗ w^{m4}/w^{m4}; +/+ ♀♀

↓

w^{m4}/Y; Df(2)/+ ♂♂

w^{m4}/Y; CyO/+ ♂♂

Figure 7. Procedure for the recovery of *In(1)wm*^{4} flies with segmental aneuploidy of the histone gene region.

Figure 8. Procedure for the recovery of *In(1)wm*^{4} flies with stable deficiencies of the histone gene region.

TABLE II. Mean Percentage of the wild-type amount of drosopterin in eyes of *In(1)w^{m4}/O; T(Y;2)/+* flies.

Second chromosome genotype of F_1 w^{m4}/O ♂♂ and breakpoints.		No. of histone gene clusters	% w+ drosopterin	$s_{\bar{y}}$
*+/T(Y;2)J59**	(43A)	2	2	0.1
*+/T(Y;2)L138**	(39C)	2	42	3.8
*+/T(Y;2)B190**	(40)	2	25	3.0
*+/T(Y;2)B209**	(40)	2	35	5.7
*+/T(Y;2)H54**	(40)	2	28	4.2
*+/T(Y;2)B26**	(43E)	2	7	1.1
*+/T(Y;2)A107**	(40)	2	47	5.6
*+/T(Y;2)B110**	(38C)	2	5	1.0
+/T(Y;2)DfB110-L138	(38C-39C)	2	7	1.7
+/T(Y;2)DfB26-J59	(43A-43E)	2	63	2.8
+/T(Y;2)DfB110-B190	(38C-40)	1	43	4.1
+/T(Y;2)DpL138-B209	(39C-40)	3	11	1.3
*+/T(Y;2)DpB110-A107**	(38C-40)	3	1	0.1
*+/T(Y;2)DpB110-H54**	(38C-40)	3	30	3.3

n $\sim$ 30 for each genotype

* Genotypes carrying B^S, expressed as % w^+ drosopterin in $+/B^SY$ heads.

phenotype by *T(Y;2)* segmental aneuploidies.

2. The Effect of Proximal (2L) Deficiencies on w^{m4} Variegation

The preceding results mandate the use of stable deficiencies, not involving altered Y chromosomes, to study the effect of histone gene multiplicity on variegation. Five proximal (2L) deficiency stocks were obtained from WRIGHT et al. (55). All have similar distal break points (Figure 1). Two of the deficiencies, *Df(2)1* and *12* extend proximally towards the histone gene cluster, but do not include it. They serve as controls for any effect of deleting euchromatic segments distal to the cluster. One deficiency, *Df(2)84*, has its proximal break point within the histone gene cluster. The proportion of the cluster which it deletes is uncertain. The remaining deficiencies, *Df(2)65* and *161*, delete the entire cluster and extend proximally. The deficiencies were tested for modification of the variegated phenotype associated with $In(1)w^{m4}$; the test scheme is displayed in Figure 8. In order to preclude any maternal effects of the deficiency, the deficiency chromosome was contributed by the male parent. A w^{m4}/Y; +/+ eye contains about 4% of the wild-type amount of the red pigment drosopterin, when flies are raised at 17°C. Heterozygosity for the control deficiencies, *Df(2)1 and 12*, and the *CyO* inversion chromosome does not significantly alter this level (Table III). $In(1)w^{m4}$ flies heterozygous for the three deficiencies which delete, all, or a part, of the histone gene cluster exhibit significantly

TABLE III. Mean percentage of the wild-type of drosopterin in eyes of *In(1)*w^{m4}/Y F_1♂flies.

F_1 ♂ Genotype	No. of Histone Gene Clusters	$\%w^+$ Drosopterin	$s_{\bar{y}}$	P
w^{m4}/Y;Df(2L)1/+	2	4	1.2	
				>0.05
w^{m4}/Y;CyO/+	2	3	0.7	
w^{m4}/Y;Df(2L)12/+	2	4	0.9	
				>0.05
w^{m4}/Y;CyO/+	2	3	0.5	
w^{m4}/Y;Df(2L)84/+	1-2	19	2.4	
				≪0.05
w^{m4}/Y;CyO/+	2	6	0.7	
w^{m4}/Y;Df(2L)65/+	1	9	2.1	
				<0.05
w^{m4}/Y;CyO/+	2	3	0.4	
w^{m4}/Y;Df(2L)161/+	1	24	3.2	
				≪0.05
w^{m4}/Y;CyO/+	2	8	1.7	
w^{m4}/Y;+/+	2	4	0.9	

elevated levels of drosopterin in the eyes. Active expression of the *white*$^{+}$ gene occurs in a larger proportion of pigment cells in w^{m4} individuals with a single histone gene cluster, than in those with a normal diploid complement.

It is possible that the observed suppression of variegation was due to peculiarities associated with the source of chromosomes of the deficiencies generated by WRIGHT et al. (55). This seems unlikely given the similar effect of deficiencies *84* and *161*, which were induced on different chromosomes. To ensure that a deletion of the histone gene cluster was responsible for the observed effect, proximal (2L) deficiencies were induced on a different chromosome, and tested using the same scheme, shown previously in Figure 8. The effect on w^{m4} variegation of the deficiencies of SINCLAIR et al. (46) was scored visually. The two deficiencies distal to, but not including, the histone gene cluster (*DS8*, *DS9*) did not enhance the proportion of cells in which the *white* gene was active (Table IV). *DS6*, which entirely deletes the cluster, and *DS5*, a partial deficiency which deletes the distal region of the cluster, caused a marked increase in pigmentation. *DS2*, a partial deficiency for the proximal portion of the cluster did not cause an increase in variegating gene activity. The obscure cytological configuration of the histone gene region makes estimation of the size of the partial deficiencies difficult.

These results contradict the hypothesis that pecularities of the source chromosome are responsible for the observed

TABLE IV. Effect of the proximal (2L) deficiencies of SINCLAIR et al. (46) on w^{m4} variegation.

GENOTPYE	NO.OF HISTONE GENE CLUSTERS	VISIBLE SUPPRESSION
Df(2)DS8/+	2	no
Df(2)DS9/+	2	no
Df(2)DS2/+	1-2	no
Df(2)DS5/+	1-2	yes
Df(2)DS6/+	1	yes

modification of variegation. However, they do suggest that a deletion of the distal portion of the histone gene cluster is necessary to elicit suppression of the position-effect.

In order to determine if the observed suppression of the position effect was restricted to males, a different cross was used to obtain $_{w}^{m4}$ females bearing the deficiencies (Figure 9). The results illustrated in Table V reveal a pattern on phenotype modification similar to males. (Control deficiency *12*, which exhibits a marginally significant difference to its *CyO* siblings, is not significantly different from the overall *CyO* value). The presence of the Y chromosome is not necessary to elicit the suppression of position-effect variegation associated with heterozygous deficiencies of the histone gene cluster.

3. The Effect of Proximal (2L) Deficiencies on Variegation Associated with $_{B}^{SV}{}_{Y}$.

The results previously tabled do not exclude the possibility that the histone gene deficiencies exert a specific influence on the *white* locus, rather than position-effect variegation in general. It was necessary to test another variegating rearrangement to demonstrate a generalized effect (Figure 10). *Bar of Stone - Variegated* (B^{SV}) was used for this purpose.

The narrow eye phenotype associated with the mutation *Bar* is the result of a tandem duplication in the X chromosome region 16A (7). The rearrangement $_{B}^{SV}{}_{Y}$ attaches this duplicated region to the heterochromatin of the Y chromosome(8).

w^{m4}/Y; CyO/Df(2)♂ ⊗ w^{m4}/w^{m4}; +/+ ♀♀

↓

w^{m4}/w^{m4}; +/Df(2) ♀♀

w^{m4}/w^{m4}; +/CyO ♀♀

Figure 9. Cross for the recovery of *In(1)w*^{m4} females with deficiencies of the histone gene region.

TABLE V. Mean percentage of the wild-type amount of drosopterin in w^{m4}/w^{m4};+/Df(2) females and their female +/CyO siblings.

Second chromosome genotype of w^{m4}/w^{m4} females	No. of histone Gene clusters	$\%w^{+}$ Drosopterin	$S_{\bar{y}}$	P
+/Df(2)1	2	3	0.8	
+/CyO	2	4	0.6	>0.05
+/Df(2)12	2	5	1.0	
+/CyO	2	2	0.4	<0.05
+/Df(2)84	1-2	24	4.4	
+/CyO	2	5	0.8	≪0.05
+/Df(2)65	1	28	3.5	
+/CyO	2	4	0.9	≪0.05
+Df(2)16I	1	12	5.3	
+/CyO	2	2	0.4	<0.05

n≃ 20 for each genotype

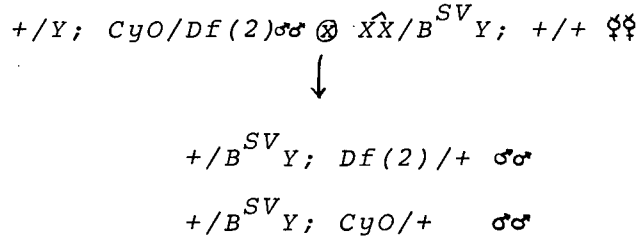

+/Y; CyO/Df(2)♂♂ ⊗ XX/B^SVY; +/+ ♀♀
+/B^SVY; Df(2)/+ ♂♂
+/B^SVY; CyO/+ ♂♂

Figure 10. Scheme for the recovery of $B^{SV}Y$ progeny which have proximal (2L) deficiencies.

As the transposed region is inactivated in a proportion of the presumptive ommatidial (eye facet) cells, B^{SV} flies exhibit a phenotype intermediate between the narrow eye of *Bar*, and the oval eye of Bar^{+}. In this case, suppression of the spreading effect should result in a narrower eye in B^{SV} flies, since the transposed duplication segment would be actively expressed in a greater proportion of presumptive ommatidial cells. This was observed in $B^{SV}Y$ flies bearing deficiencies *65,84* and *161*, confirming the generalization that heterozygous deficiencies of the histone gene cluster increase the proportion of cells in which a variegating gene is active (Table VI). The partial deficiency, *84*, had an intermediate suppressive effect on this variegating allele.

4. The Effect of Proximal (2L) Deficiencies on the Variegation of Autosomal Rearrangement *T(2;3) Stubble-Variegated*.

Since both *white* and *Bar* are X linked genes, the foregoing data would be consistent with an interpretation that the effect of histone gene deficiencies is restricted to the expression of variegating genes on the X chromosome, perhaps by a modification of the dosage compensation mechanism. This hypothesis was tested by observing the effect of the deficiencies on a variegating autosomal gene (Figure 11). The rearrangement $T(2;3)Sb^{V}$ juxtaposes the mutant allele *Sb* (which has a short bristle phene) to the heterochromatin of the second chromosome. The resultant variegation produces a mosaic pattern of short bristles (*Sb* active in the bristle-forming cells) and normal, longer bristles (*Sb* inactivated). Heterozygosity for

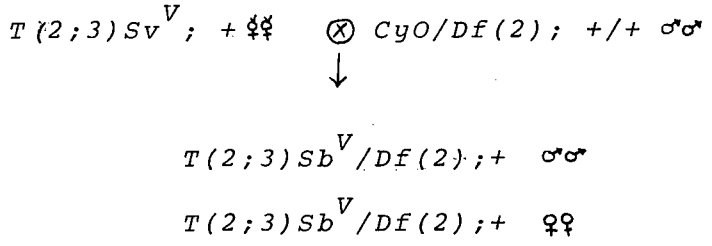

T(2;3)Sv^V; + ♀♀ ⊗ CyO/Df(2); +/+ ♂♂
T(2;3)Sb^V/Df(2);+ ♂♂
T(2;3)Sb^V/Df(2);+ ♀♀

Figure 11. Cross to generate *T(2;3)Sb*^{V} progeny with proximal (2L) deficiencies.

TABLE VI. Mean Percentage of Wild-Type (B^+) Eye Surface in $+/B^{SV}Y$ F_1♂flies.

F_1♂ Genotype	No. of Histone Gene Clusters	% B^+ Eye Size	$s_{\bar{y}}$	P
$+/B^{SV}Y;Df(2L)1/+$	2	53	1.9	
$+/B^{SV}Y;Df;CyO/+$	2	47	1.9	<0.5
$+/B^{SV}Y;Df(2L)12/+$	2	43	1.1	
$+/B^{SV}Y;Df;CyO/+$	2	48	1.2	>0.05
$+/B^{SV}Y;Df(2L)84/+$	1-2	38	1.3	
$+/B^{SV}Y;CyO/+$	2	44	1.6	<0.05
$+/B^{SV}Y;Df(2L)65/+$	1	26	0.9	
$+/B^{SV}Y;CyO/+$	2	46	1.1	$\ll 0.05$
$+/B^{SV}Y;Df(2L)161/+$	1	18	0.7	
$+/B^{SV}Y;CyO/+$	2	43	1.9	$\ll 0.05$

$n \sim 100$ for each genotype

deficiencies of the histone gene cluster (*65,84,161*) results, in a shorter mean bristle length in Sb^V flies than does heterozygosity for the control deficiencies (*1,12*)(Table VII). The variegating autosomal gene is active in a greater proportion of bristle-forming cells in those flies which have a reduced gene content than in those which have a normal complement. While this result does not preclude deficiency effects on dosage compensation, it does confirm the generalized effect of histone gene deficiency on position-effect variegation.

5. Maternal Effect on Variegation of Proximal (2L) Deficiencies

Oocytes of species which undergo rapid cellular proliferation after fertilization contain pools of histone protein and mRNA to fulfill the needs of chromosome replication (1). While it is not known whether *Drosophila* oocytes have such m-RNA pools, abundant endogenous histone protein can be extracted from early embryos (42). A histone gene deficiency in the maternal parent would result in oocytes with reduced histone mRNA or protein pools, if transcriptional capacity was a limiting factor in their production. Since such a reduction would have its effect during early development (the period when some rearrangements are sensitive to variegation modifiers), the non-deficiency progeny of histone gene deficient mothers might be expected to exhibit modification of the variegated phenotype (29). The scheme of Figure 12 was designed to test this hypothesis, by the response of $In(1)w^{m4}$ progeny to maternal histone gene deficiencies. No significant difference can be discerned between the *CyO/+* progeny of deficiency or

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

Genotype	No. of Histone Gene Clusters	% of parental *Sb*$^{+}$ bristle length and ($S_{\bar{y}}$)	
		♂	♀
T(2;3)Sb^{V}/*Df(2)1*	2	77 (3)	97 (4)
T(2;3)Sb^{V}/*Df(2)12*	2	85 (4)	90 (5)
T(2;3)Sb^{V}/*Df(2)84*	1-2	64 (3)	62 (4)
T(2;3)Sb^{V}/*Df(2)65*	1	71 (4)	74 (3)
T(2;3)Sb^{V}/*Df(2)161*	1	49 (3)	53 (5)

n$\sim$ 30 for each genotype

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

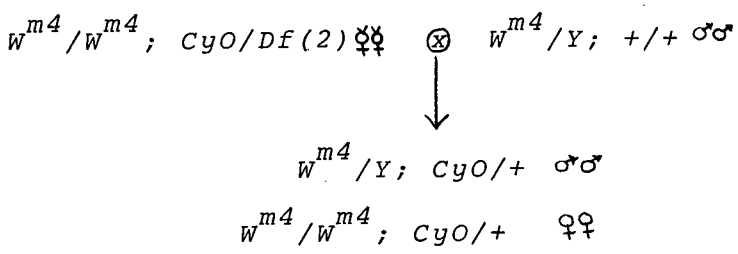

W^{m4}/W^{m4}; CyO/Df(2)♀♀ ⊗ W^{m4}/Y; +/+ ♂♂
W^{m4}/Y; CyO/+ ♂♂
W^{m4}/W^{m4}; CyO/+ ♀♀

Figure 12. Scheme to test the maternal effect of proximal (2L) deficiencies on w^{m4} variegation.

control mothers (Table VIII). Histone gene deficiencies exert no apparent maternal effect on variegation. Various hypotheses can be devised to explain this result:

(i) There are no pools of histone m-RNA or protein in *Drosophila* oocytes. This is improbable, since histone protein is abundant in the embryo, prior to the onset of zygotic transcription (42,57).

(ii) Reduction of gene multiplicity is not limiting in the production of such pools.

(iii) The transcriptional fate of the variegating gene is determined after the time at which zygotic histones supercede maternally coded histones.

All of the above hypotheses could be tested by a combination of biochemical and genetic techniques.

6. Modification of Position-Effect Variegation by Duplication of Proximal (2L).

If variegating alleles are actively expressed in a greater proportion of cells whose genotypes bear histone gene deficiencies, is the inverse observed in genotypes which bear a duplication of proximal (2L)? A procedure was devised to answer this question and to assess any maternal duplication effects (Figure 13).

Dp(2;1)C23.9 inserts all, or part, of the histone gene cluster into the euchromatin of the X chromosome (Figure 5). The intensity of in-situ histone cRNA binding to the inserted segment suggests that a majority of the histone gene cluster has been duplicated by this rearrangement. The duplication

CROSS 1	CROSS 2
$Df(2;1)C239/d149;Cy/bw^V$ ⊗ $v/Y;Cy/T(2;3)Sb^V$	⊗ $dor^1/d149;Cy/bw^V$
$DpC239/v;Sb^V/Cy$	$dor^1/v;Sb^V/Cy$
$DpC239/v;Sb^V/bw^V$	$dor^1/v;Sb^V/bw^V$
$d149/v;Sb^V/Cy$	$d149/v;Sb^V/Cy$
$d149/v;Sb^V/bw^V$	$d149/v;Sb^V/bw^V$
$d149/Y;Sb^V/Cy$	$d149/Y;Sb^V/Cy$
$d149/Y;Sb^V/bw^V$	$d149/Y;Sb^V/bw^V$

Figure 13. Crosses to test the direct and maternal effect of *Dp(2;1)C239* on variegation associated with $T(2;3)Sb^{V}$.

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

Maternal genotype	No. of maternal histone gene clusters	% w+ drosopterin and ($s_{\bar{y}}$)	
CyO/Df(2)1	2	6 (0.6)	2 (0.2)
CyO/Df(2)12	2	3 (0.4)	1 (0.2)
CyO/Df(2)84	1-2	4 (0.7)	2 (0.3)
CyO/Df(2)65	1	3 (0.4)	1 (0.2)
CyO/Df(2)161	1	3 (0.5)	1 (0.1)

$n \sim 20$ for each genotype.

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.

chromosome is lethal in the homozygous and hemizygous states. The variegated bristle phenotype associated with the rearrangement *T(2;3)Sb*^{V} was measured in females heterozygous for the duplication chromosome. It was also measured in two contols; female siblings carrying the multiple inversion X chromosome, *In(1)sc*^{S1}*+d149*, and females from another cross bearing the X chromosome point mutant, *deep-orange*^{17A}. The latter two X chromosomes did not vary in their ability to modify variegation. The results enumerated in Table IX reveal no effect on the variegated phenotype when histone gene multiplicity is increased by approximately 50%. The proportion of bristle-forming cells which exhibited inactivation of the variegating gene was not significantly different than in the controls. This result is consistent with models of histone gene activity which incorporate a maximal limit to the production of histone protein, independent of histone gene multiplicity. Alternately, a surfeit of cellular histones may have no effect on chromatin morphology, as revealed by the activity of variegating genes.

The maternal effect of the histone gene duplication could also be assessed, since identical *d149/v* and *d149/Y* progeny were obtained from both duplication or non-duplication mothers. (Figure 13). The variegated phenotype of the progeny was not significantly affected by differences in maternal histone gene multiplicity (Table IX). This result implies that the size of the oocyte pool of histone-mRNA or protein is not expanded by a 50% increase in maternal histone gene multiplicity. The result could also be explained by proposing that surplus

maternally-coded histones are not assembled into embryonic chromatin. or that such maternally-coded histones are effective prior to the determination of the transcriptional fate of the variegating gene.

TABLE IX. Mean percentage of dorso-central and scutellar bristles with a *Sb* phenotype in *T(2;3)Sb*^{V} progeny from proximal (2L) duplication and non-duplication mothers.

Cross 1 progeny genotype	No. of histone gene clusters	No. of maternal histone gene clusters	% Sb bristles	$s_{\bar{y}}$
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 progeny genotype				
dor^{1}*/v;Sb*^{V}*/Cy*	2	2	45	10
dor^{1}*/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 dorso-central bristles could be scored.

n $\sim$ 20 for each genotype.

DISCUSSION

Alteration of histone gene multiplicity has not been directly correlated to changes in the amount of cellular histone protein. It seems improbable that any reduction in histone amount is equivalent to the 50% reduction in histone gene multiplicity associated with the heterozygous deficiencies. However, the results can be interpreted to suggest that compensation for the reduction in histone coding capacity is incomplete, and that the level of cellular histone is partially reduced during the period when the transcriptional fate of variegating genes is determined. This interpretation is supported by the observation of MOTTUS et al. (40) that n-butyrate, an agent which apparently reduces histone - DNA interaction, also enhanced the activity of variegating genes.

The lack of effect of increased histone gene numbers on variegation 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 not favoured, since SPERLING and WEISS (48) 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 et al. (42) have isolated a factor from *Drosophila* embryos which 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.

The results do not entirely eliminate the possibility that the observed suppression of position - effect variegation is due to the deletion of a gene tightly linked to the histone gene cluster, rather than deletion of the cluster itself. It should be noted, however, that heterozygotes for any control deficiency distal to the cluster (*Df(2) 1,12, DS8, DS9*) do not exhibit more active expression of variegating genes than do non-deficiency controls. Flies heterozygous for deficiencies that do not extend proximally beyond the cluster (*Df(2)84,DS5*) do exhibit suppression of variegation. Hence, if deletion of a locus outside the histone gene cluster is responsible for the results obtained, it must be closely linked distal to the cluster. A recent study by HENIKOFF (26) suggests that variegation modifier loci may be as frequent as one per 25 chromomeres, and possibly are sites of non-histone chromosomal protein genes.

The partial deficiencies of the distal region of the histone gene cluster (*Df(2)84, DS5*) do not cause an intermediate enhancement of the variegating gene activity associated with w^{m4} or Sb^{V}, although an intermediate effect on B^{SV} was observed. The portion of the cluster which these deficiencies do not delete may be so small as to be inconsequential. Alternately, the portions of the cluster which they do delete may contain a subset of histone genes responsible for chromatin condensation during the period when the transcriptional fate of particular variegating genes is determined. The genes removed by *DS2*, which deletes the

proximal segment of the cluster do not have an apparent effect on the variegation of w^{m4}. These findings are consistent with a model in which the histone gene cluster is functionally differentiated. Is there evidence for differentiation among the genes for a histone protein? Is this differentiation reflected in the organization of the histone genes?

Extraction of the H1, H2A and H2B mRNAs from the blastula, mesenchyme blastula and gastrula stages of *Stongylocentrotus purpuratus* sea urchin embryos, and their translation in-vitro, yeilds stage-specific sequence variants of these proteins (52). Sequence variation is observed in the H4 mRNA from early and late developmental stages of another sea urchin, *Lytechinus pictus* (23). Histone mRNAs also vary in length during echinoderm development (23). The developmental significance of these shifts in histone gene expression has not been elucidated.

LIFTON et al. (36) reported two principal variants of the *Drosophila* histone gene repeat unit, differing by a 250 base pair insert in a non-coding spacer segment. Rare variants, containing larger inserts in spacer regions have also been observed (30). STRASBAUGH and WEINBERG (50) identified more extensive variation in repeat unit length among *Drosophila* strains and individuals. Again, the functional significance of these variants is unclear. The organization of the repeat unit variants within the histone gene cluster is unknown.

It is apparant from the occurence and temporal specificity of histone variants in sea urchin development, that subsets of the histone genes have particular developmental functions.

The nature of these functions will be elucidated only by the development of in vitro transcription systems which can assess the specific effects of histones arising from these variant sequences. The question of functional segregation of variant sequences within the histone gene cluster may yield to a genetic approach. The stage - specific effects of small, partial deletions of the cluster on phenomena influenced by chromatin morphology could be assayed. In addition to position - effect variegation, these phenomena include mitotic recombination and mutagen sensitivity (47). Biochemical parameters such as nuclease sensitivity and internucleosomal spacing could also be tested.

Certain features of the structure and organization of the histone genes have been implicated in the control of their expression. It is assumed that the arrangement of coding sequences into a unitary sequence serves to ensure coordinate transcription, since, with the exception of H1, the histones are present in equimolar amounts in the nucleosome (36). However, no polycistronic transcript corresponding to the repeat unit has been found, although high molecular weight precursors are observed in some systems (39,35). HACKETT et al. (24) concluded that polycistronic histone messengers do not exist in HeLa cells, since the induction of thymidine dimers (which act as transcription terminators) does not selectively reduce the message for any subset of the histones. In *Drosophila*, the "sense" sequences for H2B and H4 are arrayed on the complementary strand to those of the other histones, eliminating

the possibility of a single transcript for all five proteins(36).

Extensive study of the temporal activity of histone genes in somatic cells has generally revealed a close linkage to DNA replication. Histone mRNA can only be recovered from S phase somatic cell chromatin, and agents which abolish DNA replication abruptly reduce histone mRNA template activity (6,42). MELLI et al. (39) reported the presence of nuclear histone RNA transcripts made throughout the HeLa cell cycle, but DETKE et al. (15) using a more effective cell synchronization procedure, were unable to duplicate this finding. During gametogenesis, histone production is independant of DNA replication. Histones are made some weeks subsequent to the cessation of DNA replication in grasshopper spermatids and other systems of spermatogenesis (4). Histone protein and message are stockpiled during echinoderm and amphibian oogenesis for use after fertilization (1,21). The mechanism responsible for the temporal linkage of histone gene activity and DNA replication in somatic cells is unknown; however, it is interesting to note that the histone gene region is the last euchromatic segment replicated during the *Drosophila* cell cycle (20).

At the level of the individual histone gene, a "pseudogenetic" approach has probed the function of the adjacent 5' non-coding sequences (22). A cloned fragment containing a sea urchin H2A gene was subjected to restriction nuclease digestions so as to create a series of deletions of the 5' leader sequence. The deletion "mutants" were tested for

transcriptional efficiency in the *Xenopus* oocyte system. The leader sequence was revealed to have both positive and negative control functions.

In conclusion, while there is a comparative wealth of information about the sequence, organization and action of the histone genes, those features of their arrangement responsible for differential expression are, as yet, poorly characterized. It is hoped that further mutational analysis of the *Drosophila* histone gene cluster will identify those components which control its expression during the cell and developmental cycles.

SUMMARY

Histone proteins are responsible for the compaction of DNA into the primary structural unit of chromatin, the nucleosome, and act as non - specific repressors of transcription. The arrangement of histone genes is similar in most species, but little is known about the structural features which control their expression. The effect of altered histone gene multiplicity has been assayed by monitoring modification of a phenomenon which is sensitive to change in chromatin morphology:position-effect variegation. In *Drosophila*, heterozygosity for a deficiency which removes the histone gene cluster results in an increase in the proportion of cells in which a variegating locus is transcriptionally active. This effect is observed in males and females, and applies to variegating alleles throughout the genome. The results are consistent with a hypothesis which predicts that a reduction in the amount of cellular histone protein would cause a structural modification of the chromatin at the variegating locus.

Duplication of the histone gene cluster does not affect variegation, suggesting an upper limit to the production of histone protein, independent of gene multiplicity. Neither increase, nor reduction of the histone gene number in the maternal genome altered the variegated phenotype of the progeny. Therefore, the effect is not oocyte transmissible.

Partial deficiencies of the histone gene cluster do not have consistent effects on the modification of variegation,

implying a functional heterogeneity within the cluster, as has been proposed for other systems.

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