THE EFFECT OF CHROMATIN STRUCTURE ON P ELEMENT-

INDUCED MALE RECOMBINATION IN

DROSOPHILA MELANOGASTER

BY

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ABSTRACT

Dysgenic male recombination (MR) induced by the P strains
T-007 and OK1 rarely, if ever, occurs in the heterochromatin
of chromosome two. One possible explanation is that the lack
of heterochromatic exchange is due to the highly condensed
chromatin in this region. Butyrate (a suspected modifier of
chromatin structure) induced significant levels of
heterochromatic MR in dysgenic hybrids derived from crosses
involving two different P strains. This finding is consisten
with the hypothesis that chromatin structure can influence the
insertion and excision of P elements and hence MR. Analogous
experiments were performed using third chromosome suppressor
of variegation (Su(var)) mutations. Neither suppressor
mutation induced any heterochromatic MR, suggesting that the
mode of action of these Su(var) genes is different from, and
more specific than, that of butyrate. One of the mutations
(325) which is thought to influence meiotic recombination
frequencies, causes some alterations in euchromatic MR in
crosses involving the OK1 strain. The other mutation, 318,
affects neither meiotic nor dysgenic recombination. Su(var)
325 is the first known "factor" to influence meiotic and
dysgenic recombination similarly.

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INTRODUCTION

While looking for segregation distortion effects in wild caught strains of <u>Drosophila melanogaster</u>, Hiraizumi (1971) noted low but significant levels of male recombination. This finding contradicted the long established belief that meiotic recombination did not occur in male <u>D. melanogaster</u>. Hiraizumi's observation immediately prompted the extensive investigation of the special circumstances under which MR can arise. Since the initial reports (Hiraizumi, 1971; Hiraizumi <u>et al.</u>, 1973), male recombination among the progeny of wild caught <u>Drosophila melanogaster</u> individuals has been observed and studied by numerous investigators: Voelker, 1974; Waddle and Oster, 1984; Sved, 1974; 1976; Cardillino and Mukai, 1975; Kidwell and Kidwell, 1975; Yamaguchi, 1976;Yannopoulos and Pelecanos, 1977; Woodruff and Thompson, 1977; Green, 1977; Golubovsky <u>et al.</u>, 1977. In all cases the recombination was observed in male hybrids derived from crosses between male parents from recently wild caught strains and female parents from established laboratory strains. However, male recombination is only one of several traits diagnostic of a syndrome termed hybrid dysgenesis (Kidwell <u>et al.</u>, 1977).

Hybrid dysgenesis (reviewed in Bregliano <u>et al.</u>, 1980; Green, 1980; Bregliano and Kidwell, 1983; and Engels, 1983)

consists of a set of genetic and physiological abnormalities which include the induction of single site visible and lethal mutations, chromosome rearrangements (Berg et al., 1980; Yannopoulos et al., 1983), mitotic recombination in females (Slatko, 1978; Sinclair and Green, 1979) as well as males, chromosomal non-dysjuction, unequal transmission of homologous chromosomes, and gonadal dysgenesis (G.D.) sterility. These abnormalities seem to be restricted to the germ line and occur before and/or during meiosis.

Hybrid dysgenesis occurs only in the F1 offspring resulting from matings between males from wild populations and females from laboratory strains. Progeny from the reciprocal cross (laboratory males crossed to wild caught females) display the traits at a frequency at least ten-fold lower than do those from the dysgenic cross. Intra-strain crosses rarely if ever produce dysgenic offspring. Thus, wild caught strains were named P (for paternal), and laboratory strains M (for maternal).

Early speculations into the etiology of the dysgenic anomalies included: infectious agents such as viruses causing random chromosome breakage (Sved, 1978), single locus mutator effects, and chromosomal-cytoplasmic interactions (Sved, 1976). The latter is consistent with the non-reciprocal nature of the syndrome. To produce the dysgenic symptoms, P chromosomes must be placed in an M-type cytoplasm. In theory

some agent (or the lack thereof) in the M cytoplasm interacts with some component or product of the P chromosomes. The nature of the "agents" or "components" was vague, although there was some suggestion (Engels, 1979) of an analogy between hybrid dysgenesis and zygotic induction of bacteriophage lambda. Single locus effects were ruled out when major "mutator" elements could not be accurately mapped to any single chromosomal position (Slatko and Green, 1980).

A pivotal observation which helped to promote the infectious agent or viral hypothesis was the finding that in hybrids P chromosomes could "contaminate" M chromosomes in a matter of several generations, i.e. the M chromosomes took on P-like characteristics and could produce dysgenesis when introduced back into the original M strain (see Bregliano and Kidwell, 1983). However, viruses were not observed consistently in P strains and furthermore, the virus theory alone could not explain the non-reciprocal aspect of the interaction.

Despite the fact that male recombination (MR) was the first known indicator of dysgenesis, the emphasis soon shifted to the investigation of mutation induction, a somewhat more easily observed aspect of dysgenesis. Early insights into the nature of dysgenically induced mutations came from Green (1977). He had observed that visible dysgenic mutations

behaved like mutable white ($\underline{w}$) alleles in that they displayed a high degree of instability. Mutations could revert to wild type or, alternatively, to a stable mutant state. Based purely on these genetic data, Green suggested that the mutations observed in dysgenic hybrids were insertion mutation similar to those found in prokaryotes (Green, 1976). He postulated that the abnormal traits associated with hybrid dysgenesis resulted from the transposition of a mobile element associated with P chromosomes. More specifically, he proposed that single site mutations were the consequence of the element inserting into specific genetic loci, and that reversions could occur upon removal of the elements by subsequent transposition or excision events.

Green's hypothesis has been confirmed through molecular analysis (Collins and Rubin, 1982; Rubin, Kidwell, and Bingham, 1982; Bingham, Kidwell, and Rubin, 1982; O'Hare and Rubin, 1983; reviewed in Engels, 1983). Dysgenically induced $\underline{w}$ mutations were shown to be associated with the presence of inserted foreign DNA into the locus. Reversion of those mutations which were unstable was invariably accompanied by the excision of most or all of the insert. The inserted elements were named P elements. All P strains have been found to carry P elements: about 30-50 per genome, scattered over all major chromosomes. Most M strains lack P elements; all M strains lack functional P elements. Because the elements are

apparently non-mobile in the P strain, it is believed that P strains produce a repressor that accumulates cytoplasmically. M strains lack both the transposable element and the repressor. The absence of the repressor in hybrids between P males and M females allows mobility of the element. This system is consistent with the various properties of hybrid dysgenesis, although a repressor has not been isolated.

The P element has been isolated and characterized (O'Hare and Rubin, 1983). The complete element is 2.9 kb in length, with 31 bp terminal inverted repeats and contains 4 open reading frames. Incomplete or defective P elements exist. All of these contain internal deletions. For example, defective elements associated with mutations at the white locus (O'Hare and Rubin, 1983), ranged in size from 0.5 to 1.6 kb in length. Incomplete P elements are mobile only so long as they are accompanied by complete elements. Thus, it appears that the open reading frames in the complete element encode a product required for transposition. This product has been termed "transposase". Frameshift mutations in any of the four reading frames of the complete element abolish the transposase function. In addition, pairwise combinations of these mutations fail to restore this function. It is therefore believed that all four reading frames are involved in the production of a "transposase" (Karess and Rubin, 1984).

As noted previously, a similar analysis of the proposed "repressor" function has not yet been possible.

The sites of P-induced rearrangements (thought to reflect sites of P element residence) are non-random and specific "hot-spots" exist (Engels and Preston, 1981). A recognition sequence into which the P element inserts is 8 bp long, and this sequence is duplicated upon insertion of the P element into a new chromosomal location. The existence of a particular recognition sequence might contribute to the apparent site-specificity exhibited by the traits of dysgenesis, such as chromosome rearrangements (Simmons and Lim, 1980). The consensus sequence for P element insertion is -GGCCAGAC- (O'Hare and Rubin, 1983). Although a preferred insertion site exists, its sequence is not highly conserved. Thus, it is likely that controls other than a recognition sequence can influence the position at which insertion occurs.

Although the nature of dysgenically induced mutation is now well understood, the mechanism by which transposable elements cause recombination remains unclear. Male recombination is not influenced by those factors known to alter meiotic recombination (Engels, 1983). Recombinant events observed are clustered and frequently only one of the two possible reciprocal products is recovered (due to the premeiotic timing of the exchanges). Several studies provide evidence that P-mediated crossover events are symmetrical

exchanges (Voelker, 1974; Sved, 1978; Isaakson, Johnson and Denell, 1981; Sinclair and Grigliatti, 1985) and thus not the products of random breakage which would be expected to produce small deletions and duplications. The pattern of recombination is non-random, and does not reflect the meiotic pattern normally observed (Engels, 1983). The pattern of recombination differs from strain to strain as do the sites of residence and thus, recombination may be related to the sites of residence of the elements. In an extension of the rearrangement hypothesis of Engels and Preston (1984), it has been suggested that DNA strand breaks at the termini of P elements may result in host strand infiltration of the DNA duplex of the closely paired homologous chromosome, leading to precise recombination (Sinclair and Grigliatti, 1985).

A number of studies have shown (Hiraizumi et al., 1973; Woodruff and Thompson, 1977) that recombinant events occur preferentially in the intervals nearest the centromere. This led to the suggestion that the majority of the recombinant breaks took place in centric heterochromatin. However, this could not be verified because appropriately marked chromosomes did not exist. In each case examined, the intervals with disporportionately high levels of MR contained both euchromatin and heterochromatin. Therefore it could not be established whether the recombination took place

preferentially in one or the other chromatin type.

Sinclair and Grigliatti (1985) utilized a specially constructed second chromosome to examine the pattern of dysgenic recombination induced by four P strains: h12, T-007, OK1, 1978. They used the heterochromatic markers light (lt) and rolled (rl) to delineate a region of the chromosome which is entirely heterochromatic. Male recombination in euchromatic segments was observed in all strains. However, in experiments involving 3 of the 4 strains, no recombination was observed between the heterochromatic markers. T-007, OK1, and 1978 each showed high frequencies of MR in the regions flanking the centric heterochromatin but none actually occurred in the heterochromatin between lt and rl. In contrast, in the experiment involving h-12, heterochromatic crossing over was observed, but at a very low frequency. The patterns of recombination observed were strain specific and different from that obtained by irradiation. Sinclair and Grigliatti attribute the strain specificities to differences between the strains in the sites of residence of P elements. They argue that the extremely low incidence of heterochromatic recombination might result from the absence, or from a low frequency of the P recognition sequence, or from the inability of the P element to insert into heterochromatic regions due to steric constraints.

Chromatin structure can influence gene activity. For

example, transcriptionally active genes are associated with chromatin in which the core histones - especially H3 and H4 - are hyperacetylated (Davie and Candido, 1978; Levy-Wilson et al., 1979). The acetyl groups on the histones may interfere with and thus weaken the histone-DNA interaction in the typical nucleosome core structure and this may lead to a more open, loose chromatin structure. Possibly this "open" conformation facilitates the binding of RNA polymerases to the DNA, thereby promoting transcription.

By analogy, one could argue that chromatin structure also influences P element insertion. Thus, although recognition sequences may exist in both heterochromatin and euchromatin, regions of the DNA in the former may be so highly condensed that they are essentially inaccessible to the P elements. Therefore, one might expect little if any dysgenic recombination in heterochromatin because it is highly condensed. One prediction of this hypothesis is that alterations in chromatin structure might allow P insertion in different areas of the genome and so may alter the pattern of dysgenically induced recombination. Most notably, recombination would be expected in heterochromatin where none had been previously observed, although changes might also occur in euchromatic intervals.

One possible means of altering chromatin structure is

through treatment with n-butyrate. In HeLa cells, n-butyrate induces a rapid and extensive increase in the amount of acetylated histones (Riggs et al., 1977; Vidali et al., 1978; Simpson, 1978; Candido et al., 1978; Sealy and Chalkley, 1978; Cousens et al., 1979; D'anna et al., 1980). This effect appears to be due to non-competitive inhibition of the deacetylase enzyme (Reeves and Candido, 1978; Boffa et al., 1978; Vidali et al., 1978), rather than to increased rates of acetylation. In addition to these observations, it has been established that butyrate treatment induces new protein and mRNA synthesis in Friend cells (Reeves and Cserjesi, 1979). Changes in the patterns of transcription due to butyrate treatment have been noted (Tralka et al., 1979; Griffen et al., 1974; Leder and Leder, 1975; Prasad and Sinha, 1976; Prasad, 1980; Chou et al., 1977; Tallman et al., 1977; Ghosh and Cox, 1977; Leavitt and Moyzis, 1978; Fallon and Cox, 1979; Rubenstein et al., 1979; Hayman et al., 1980). Butyrate also reverses malignant transformation, yielding cells with normal morphological and biochemical characteristics. Moreover, it renders chromatin more accessible to nucleases such as DNAase I and II, and micrococcal nuclease (reviewed by Kruh, 1982). The effects of butyrate on cells in culture are reversible. In addition to the effects on cell culture, in vivo studies have demonstrated that butyrate can inhibit phosphorylation of histones H1 and H2A and impair methylation of arginine and

lysine residues in histones and other nuclear proteins (Boffa et al., 1981).

An important phenomenon relevant to the discussion of in vivo effects of butyrate on gene expression is that of position effect variegation in Drosophila melanogaster. Position effect variegation is the mosaic expression of a gene resulting from its proximity to a broken piece of heterochromatin. The gene may be active in some cells and not in others. Often the transcriptional fate of a variegating gene is determined early in development and is clonally propagated. Most loci examined have been found to variegate, and in the majority of cases a variegating system involves a euchromatic gene or genes juxtaposed to a centric heterochromatic segment. In cases where the activities of several loci are detectable, loci closest to the rearrangement breakpoint are most likely to be inactivated (Hartmann-Goldstein, 1967). In a homozygote, the inactivation of each locus is an independent event. That variegation is not the result of any permanent alteration or mutation in the gene can be shown by recombination experiments (for review, see Spofford, 1976).

In many respects, variegation resembles X-chromosome inactivation in mammals. It is thought to result from "heterochromatic spreading" (first observed by Demerec and

Slizynska, 1937): the progressive condensation or heterochromatinization of the chromatin, extending distally from the rearrangement breakpoint to a greater or lesser extent in individual cells. There is a good correlation between cytological observations on the number of cells in which a particular locus is heterochromatinized and the degree of mosaicism in an individual's phenotype (Hartmann-Goldstein, 1967).

It has been shown (Mottus et al., 1980, 1982), that sodium butyrate effectively suppresses the inactivation of w^+ in w^{m4} individuals and (Moore et al., 1979, 1983) that reductions in histone gene multiplicity also suppress variegation. Thus reductions in the number of histones present or in the affinity of histones for DNA alter chromatin structure to the extent that variegation (heterochromatization) is affected.

In summary, butyrate is associated with certain chemical changes which are thought to result in altered chromatin structure. In addition, it is known to suppress the inactivation of euchromatic genes near a heterochromatic breakpoint. It is believed that this effect results from the inability of heterochromatin to "spread" over the euchromatic region (due to the loosening of its chromatin structure). Because of this suspected action of butyrate upon heterochromatin, it was used in this study to treat dysgenic

hybrids of two P strains. If butyrate reduces the hypothesized steric constraints upon P element insertion in heterochromatin one would expect to observe heterochromatic male recombination as a result of the butyrate treatment.

Chemicals added to the culture medium of any organism may produce multiple effects. In the case of butyric acid, altered chromatin structure may be only one of them. Variegation suppressor genes, thought to be involved in the assembly or maintenance of chromatin structure, offer the opportunity to disrupt chromatin structure in a more precise way, without the side effects which may be produced by exogenous chemical treatments.

Using w^{m4} variegation as an assay, Sinclair et al. (1983) isolated 51 dominant variegation suppressor (Su(var)) and 3 variegation enhancer (En(var)) mutations in Drosophila melanogaster. The majority of the Su(var) genes are clustered on chromosomes 2 and 3. Su(var) mutants suppress several different variegating systems. Because of this apparent general effect on variegating systems, it is argued that the products of Su(var) genes are involved in chromatin structure; perhaps as structural, non-histone chromosomal proteins or enzymes for assembly, modification or maintenance of chromatin.

One of the clusters of Su(var) genes on the third

chromosome is located near an unusual pair of sites at 87B-E (Henikoff, 1979). Deletion of these sites results in suppression of variegation. Sinclair et al. therefore suggest that many of the Su(var) mutations may be hypomorphs or amorphs. There is some indication that these genes normally code for products associated with heterochromatin. For instance in males in which the Y chromosome is absent, the level of suppression of the Su(var) mutations is substantially reduced. The Y chromosome is almost entirely heterochromatic; the implication is that its absence frees up an otherwise very limited level of product which can then participate in the heterochromatic spreading, inactivating the variegating locus. Also, cytological evidence (Hayashi, unpublished) indicates that the variegating white gene is more often euchromatic in the presence than in the absence of the suppressor mutations. Biochemical evidence (Ruddell, in preparation) indicates that the variegating $\underline{w}$ gene of $\underline{w}^{m4}$ alters chromatin structure (DNAase sensitive sites and nucleosome spacing) and that at least some suppressor genes return the chromatin structure to that observed for wild type flies.

In principle, a suppressor mutation such as those described above could be used to alter chromatin structure (perhaps specifically that of heterochromatin, in contrast to the general effect of butyrate), without the complication of the possible side effects of chemical treatments.

Accordingly, dysgenic and non-dysgenic crosses were performed as in the butyrate experiments with the addition of one of two third chromosome Su(var) genes chosen for the strength of their suppression of variegation.

MATERIALS AND METHODS

Culture conditions:

Flies were reared in half pint milk bottles or 8 dram shell vials on a standard sucrose-cornmeal-agar medium. Tegosept (methyl-p-hydroxybenzoate) was included in the food as a mould inhibitor. A combination of ampicillin with either streptomycin or tetracycline was used (10 mg/l each) to suppress bacterial growth. Where noted, some crosses were raised on food to which butyric acid was added to a final concentration of 150 mM. This concentration was used to maximize the effect on chromatin structure while minimizing the delay in development which is observed as a result of butyrate treatment (Mottus, 1979). In all cases, the final pH of the food was adjusted to 5.5. All crosses were performed at 22°C.

P-Strains and marked chromosomes:

The P-strains used were: T-007/CyO (T-007 isolated in Texas, Hiraizumi, 1971; Hiraizumi et al., 1973) and OK1/OK1 (OK1 isolated in Oklahoma, Woodruff and Thompson, 1977). The highly inbred laboratory strain Oregon R was used in place of the P strains in the control crosses (Oregon R is an M strain).

The strain used to monitor recombination was an M strain

bearing the second chromosome: Star ($\underline{S}$, genetic position 1.3), black ($\underline{b}$, 48.5), light ($\underline{lt}$, 55.0), rolled ($\underline{rl}$, 55.1), narrow wing-dominant ($\underline{nw}^D$, 83.0) and Pin-yellow tip ($\underline{Pin}^{Yt}$, 107.3). For a description of these mutations and their phenotypes, see Lindsley and Grell (1968; see also Sinclair and Grigliatti, 1985). The multiply marked chromosome can be divided into five intervals (see Figure 1). Note that the interval between $\underline{lt}$ and $\underline{rl}$ (interval number 3) is entirely heterochromatic, whereas intervals 2 and 4 span both heterochromatin and euchromatin, and intervals 1 and 5 are entirely euchromatic. This chromosome was maintained in culture as a heterozygote with the multiply inverted chromosome: $\underline{In: (2L, 2R) CyO}$ (hereafter referred to as $\underline{CyO}$).

The M strain used in the suppressor crosses was the same, with the addition of a third chromosome homozygous for either the gene $\underline{Su(var)325}$, or $\underline{Su(var)318}$; hereafter referred to as $\underline{325}$ and $\underline{318}$ (described in Sinclair et al., 1983).

Genetic crosses:

The procedure for the untreated and butyrate treated crosses is outlined in Figure 2(a).

(a) Standard recombination experiments: $\underline{T-007}$ or $\underline{OK1}$ males were crossed to multiply marked M strain females. Approximately 25 flies of each sex were placed in half-pint milk bottles on normal food and allowed to lay eggs for three
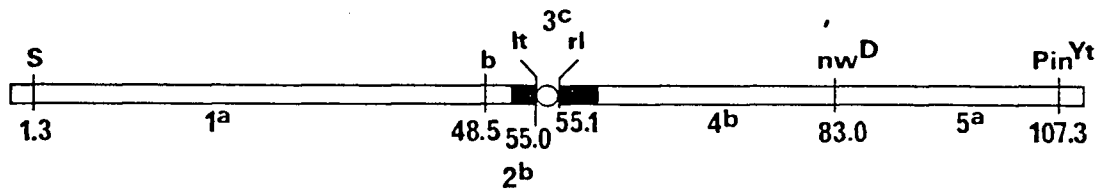
Figure 1. Multiply marked second chromosome used in all experiments.

    a   interval entirely euchromatic

    b   interval spans both euchromatin and heterochromatin

    c   interval entirely heterochromatic

A   Untreated and butyrate treated crosses

P $\dfrac{\text{S b lt rl nw}^D \text{Pin}^{Yt}}{\text{CyO}}$ ♀♀ (X) $\dfrac{\text{T-007}}{\text{CyO}}$ or $\dfrac{\text{OK1}}{\text{OK1}}$ or $\dfrac{\text{OreR}}{\text{OreR}}$ ♂♂

Half the parents lay eggs on
standard medium, the other
half on butyrate treated
medium

F1 $\dfrac{\text{S b lt rl nw}^D \text{Pin}^{Yt}}{\text{T-007 or OK1 or OreR}}$ ♂♂ (X) $\dfrac{\text{b lt rl}}{\text{b lt rl}}$ ♀♀

F2   Progeny scored for recombination on chromosome 2

B   Suppressor crosses

P $\dfrac{\text{S b lt rl nw}^D \text{Pin}^{Yt}}{\text{CyO}}$; $\dfrac{\text{Su(var)}}{\text{Su(var)}}$ ♀♀ (X) $\dfrac{\text{T-007}}{\text{CyO}}$ or $\dfrac{\text{OK1}}{\text{OK1}}$ or $\dfrac{\text{OreR}}{\text{OreR}}$ ♂♂

F1 $\dfrac{\text{S b lt rl nw}^D \text{Pin}^{Yt}}{\text{T-007 or OK1 or OreR}}$; $\dfrac{\text{Su(var)}}{+}$ ♂♂ (X) $\dfrac{\text{b lt rl}}{\text{b lt rl}}$ ♀♀

F2   Progeny scored for recombination on chromosome 2

Figure 2.   Mating scheme used in untreated and buyrate-
treated (A) and Su(var) (B) experiments. Except
where noted, all crosses were raised on standard
medium.

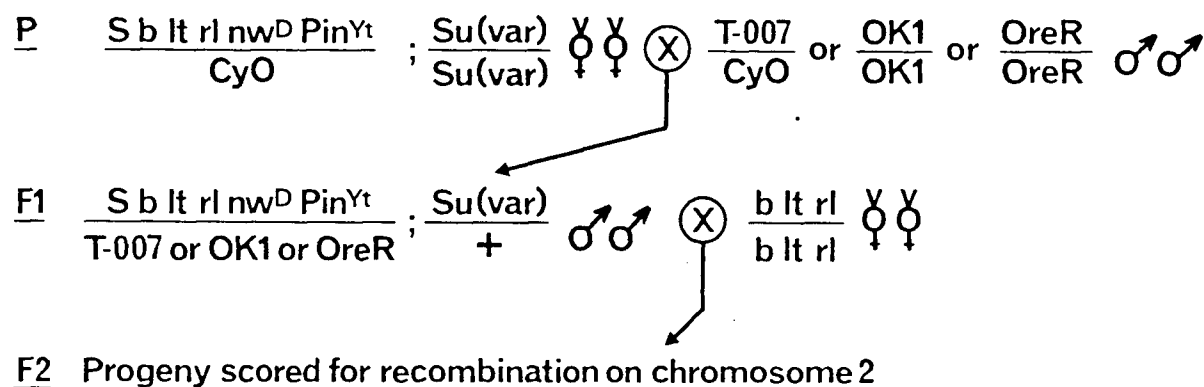days. The parents were transferred to fresh bottles and allowed to lay eggs for three more days, and were then discarded. At least 5 bottles were set up for each of the P strains. F1 hybrid male progeny bearing the multiply marked second chromosome and a second chromosome from the P strains were collected and then back crossed to virgin b lt rl females. In each case 3 males were mated with 10 virgin females in 8 dram shell vials. For each cross at least 50 vials were set up. The parents were allowed to lay eggs for three days and the males were then transferred to new vials with 10 new females. The original females were transferred to a half-pint milk bottle and allowed to continue to lay eggs for 5 more days. This process was repeated twice to provide three replicates for a total of at least 150 vials for each cross. Progeny from these crosses were examined for recombination on the second chromosome for 21 days after the parents were introduced into the bottles or vials. As a control for these crosses, the same procedure was followed except that instead of a P strain, males from the Oregon R strain were used.

(b) Butyrate recombination experiments: The parents for these experiments were obtained from the same cultures as those used in the aforementioned study. The butyrate crosses were performed essentially as described above, except that the

F1 individuals were grown on medium containing butyrate (150mM).

(c) Suppressor experiments: The protocol for the suppressor crosses is given in Figure 2(b). Two Su(var) mutations were tested for their effect on MR by procedures analogous to those described above. In this case T-007, OK1 or (for a control) Oregon R males were crossed to females bearing the multiply marked second chromosome $\underline{S}$ $\underline{b}$ $\underline{lt}$ $\underline{rl}$ $\underline{nw}^D$ $\underline{Pin}^{Yt}/\underline{CyO}$ and a third chromosome carrying one of two Su(var) mutations: either $\underline{Su(var):325}$ or $\underline{Su(var):318}$. Each population of either the P strain or the Oregon R males was divided in half so that one half of the group was used in the 325 cross and the other in the 318 cross. Thus, the parents in the 325 crosses were from the same cultures as those used for the 318 crosses.

Analysis of the data:

Because the exchange events occur premeiotically, it is possible for the products of a single exchange to be propagated through subsequent mitotic divisions prior to meiosis. Both products of exchange may be represented in the progeny, or large numbers of progeny bearing one or the other product may be observed. Most often, a single product of exchange is represented in one of the offspring. When a single F2 individual bears a single product of exchange, it is

clear that one exchange event (in the regions of interest) has taken place. When both reciprocal products are observed, either singly or in groups, or large groups of a single product are observed (either of these possibilities falls under the term "cluster"), it is clear that at least one event has taken place, but there is a small possibility that there has been more than one exchange. In analysing the data generated in this study, it was of less interest to know the total number of recombinant individuals than the number of recombinant events which took place; each of which, directly or otherwise, represents the action of one P element. Because the F1 males were mated in groups of three (high levels of sterility observed prior to these experiments made mating males individually impractical), it is impossible to tell whether all members of a cluster of recombinant progeny originated from the same parent. It was therefore necessary to estimate the number of single events which occurred. This was done by considering each cluster which occurred to be the product of a single event.

In Tables 1-4, the number of estimated single events is shown for each interval on chromosome two as well as the total estimated single events. On the line below, in parentheses, is given the total number of recombinant individuals observed. This allows some estimation of the degree of clustering for each interval. In the results section, the number and percent

of clusters are given for each experiment. This information was included because it may indicate whether the butyrate treatments or suppressors had any effect on the timing of the exchange events, which would be expected to show up in the proportion of recombinant events which were clustered. The estimated number of single events is necessarily an underestimate, since in a small proportion of cases, a cluster may contain the identical products of two separate exchange events (that is, from two separate parents), but this is a very minor source of error.

The data were analysed for statistical significance using the G test (Sokal and Rohlf, 1969) at the standard p=.05 level of significance.

RESULTS

(a) Standard recombination experiments

Meiotic recombination does not occur in Drosophila melanogaster males that lack P elements. To demonstrate this general absence of meiotic recombination, Oregon R males (M strain) were mated with females bearing the multiply marked second chromosome and their male F1 offspring, heterozygous for the multiply marked second chromosome were backcrossed to b lt rl virgin females. As expected, there were no recombinants among the 7,919 F2 offspring examined (data not shown).

The male recombination data for the T-007 and OK1 experiments are presented in Table 1. In both cases, substantial recombination was observed among male hybrids resulting from P male and M female matings. The T-007 and OK1 untreated crosses each produced a characteristic pattern and frequency of recombination. The frequency of recombination events among the T-007 males was 1.15%, whereas the frequency among the OK1 strain males was only 0.74 percent. The number and frequency of recombinants that occurred in each interval of the chromosome for the T-007 and OK1 experiments are also given in Table 1.

P Strains		Genetic Interval					Total	Flies Scored
		1	2	3	4	5		
T-007	number[a]	25 (27)	33 (67)	0	34 (82)	24 (34)	116 (220)	10,087
	per cent[a]	.248 (.367)	.327 (.664)	0	.337 (.813)	.238 (.337)	1.15 (2.18)	
	distribtion[b]	.216	.285	0	.293	.207	1.000	
OK1	number[a]	14 (23)	13 (49)	0	20 (63)	11 (18)	58 (152)	7,885
	per cent[a]	.178 (.292)	.165 (.621)	0	.254 (.786)	.140 (.228)	.736 (1.93)	
	distribution[b]	.241	.224	0	.345	.190	1.000	

Table 1.   Recombination data, in each genetic interval, for control dysgenic crosses involving P strains T-007 and OK1.

a   data for recombination events given first with total recombinants given in parentheses (see "Materials and Methods").

b   data based on recombination events only.

Sinclair and Grigliatti (1985) found that of the four P strains they examined, T-007 was the most potent inducer of MR. This study confirms this observation. The data for the OK1 strain in both studies are nearly identical in both pattern and frequency of recombinant events. The frequency and pattern of events for the T-007 strain observed in this study showed one major difference from that of Sinclair and Grigliatti. In every recombinant interval as well as in total, the frequency of events observed was consistently two-thirds that observed in the previous study. Perhaps this can be explained by the fact that the T-007 culture used in this study was derived from a single bottle of the culture used by Sinclair and Grigliatti. There may be some variation in the stock such that the proportion used to found my culture was not representative of the entire population. Alternatively, small variations in laboratory conditions, such as temperature, may have influenced the levels of recombination induced by T-007, although it is not clear why one P strain and not the other would be sensitive to these slight variations. In any case, the general trends observed for T-007 were similar for both studies. The highest levels of MR were always observed in intervals 2 and 4; the intervals containing both euchromatin and heterochromatin. Most importantly, for both studies, there was no heterochromatic

recombination observed for either of the strains. In addition, a comparison of recombinant frequencies based on events (data corrected to account for clustering) showed much greater similarity between the two studies than that based on the number of recombinant individuals observed. Since the studies were practically identical in procedure, this may be taken as an indication that the corrected data represent the more accurate estimate of recombination frequency.

Despite the fact that the two P strains each induce a characteristic pattern and frequency of recombination, there were some interesting similarities between the two. The most important observation (as noted above) is that no recombination occurred in interval number 3; the only wholly heterochromatic interval, in either the T-007 or the OK1 strains. In both strains, a substantial proportion of the total recombination (approximately 60%) occurred in intervals 2 and 4. These intervals are both adjacent to the centromere and are comprised of both euchromatin and heterochromatin. While the majority of the recombination was observed in these two intervals, the pattern was somewhat different between the two strains.

(b) Butyrate recombination experiments

To test the effect of butyrate on male recombination the

same crosses as in the untreated set were performed and the F1 hybrid males were raised on mediume to which butyric acid had been added to a concentration of 150mM. Treatment with butyrate in the absence of P elements (in the Oregon R cross) had no effect on male recombination. No recombinants were discovered among the 10,462 F2 progeny of the Oregon R crosses. Therefore, it can be concluded that butyrate alone does not induce perceptible levels of male recombination.

In contrast, butyrate treatment altered the male recombination events which occurred in the T-007 and OK1 crosses. In the T-007 cross (Table 2), the overall recombination (event) frequency increased significantly from 1.15% to 1.49% (G test, p=.05). Moreover, the pattern of recombination was altered by butyrate treatment. Most importantly, a low, but perceptible level of recombination (.048%) was observed for the heterochromatic interval. This effect is statistically significant (using the G-test, Sokal and Rohlf, 1969). Recall that recombination is never observed in this interval in the absence of butyrate. In addition, T-007-induced exchange on the right arm of chromosome two (intervals 3, 4, and 5) showed a statistically significant increase relative to comparable control levels. On the other hand, intervals 1 and 2 (on the left arm) were unaffected by the butyrate treatment. Finally, a small increase in the number of premeiotic exchange events as shown by a slight

P Strains		Genetic Interval					Total	Flies Scored
		1	2	3	4	5		
T-007	number[b]	30 (45)	48 (77)	7 (7)	74 (221)	56 (87)	215 (437)	14,453
	per cent[b]	.208 (.311)	.332 (.533)	.048 (.048)	.512 (1.53)	.388 (.602)	1.49 (3.02)	
	distrib- tion[b]	.140	.223	.033	.344	.261	1.000	
OK1	number[b]	13 (22)	34 (78)	4 (4)	50 (106)	17 (44)	118 (254)	16,803
	per cent[b]	.077 (.131)	.202 (.464)	.024 (.024)	.298 (.631)	.101 (.262)	.702 (1.51)	
	distri- bution[c]	.110	.288	.034	.424	.133	1.000	

Table 2. Recombination data, in each genetic interval, for butyrate[a] treated dysgenic crosses involving P strains T-007 and OK1.

a   .15M

b   data for recombination events given first with total recombinants given in parentheses (see "Materials and Methods").

b   data based on recombination events only.

increase in the percent of clusters was observed. In the untreated (or standard) experiment, 29 of the 116 recombination events observed appeared as clusters. This represents 25% of the events observed. In the butyrate experiment 78 of 215 events observed were clustered, which corresponds to 36 percent of the events observed. This increase is not statistically significant.

In contrast with the T-007 strain, the overall frequency of recombination events induced by the OK1 strain remained unaltered (about 0.8% for both treated and untreated crosses) by butyrate treatment. However, as with the T-007 strain, the pattern of recombination events was altered by exposure to butyrate. Most notably, a small (0.24%), but significant amount of recombination occurred within the centromeric heterochromatin of region 3. In contrast, there was a significant decrease in exchange frequency for region 1, whereas for intervals 2, 4 and 5, the frequency of recombination events remained at about the same level as in the untreated crosses. There was no appreciable change in the percent of clusters produced in response to butyrate. In the untreated, or standard, experiment, 20 of 58 observed recombination events were clustered (34%). In the butyrate experiment, exactly the same percent of the events was clustered (40 of 118 events).

One of the most important effects of butyrate is the

occurrence of recombination in the heterochromatin, where it has not previously been observed. In the case of both T-007 and OK1, all of the heterochromatic exchange events observed were single, unclustered events. Based on the data for all of the crosses, an average degree of clustering of 32% can be obtained. Given this, and if it is assumed that the timing and nature of the heterochromatic events is the same as those of the euchromatin, 4 of the 11 heterochromatic crossovers could have been expected to be premeiotic and as such would have appeared as a cluster of recombinants. The absence of clusters may reflect the small sample size, or it may be an indication of an important difference in heterochromatic vs. euchromatic crossing over.

(c)   Su(var)325

Preliminary mapping experiments suggest that the Su(var) 325 mutant may influence meiotic crossing over (Tejani and Kroitsch, 1982, personal communication). Since many of the basic processes which occur in meiotic crossing over are probably important for male recombination, it was of interest to determine whether the suppressor 325 could influence dysgenic crossing over. Also, since suppressors of variegation and butyrate have the same effect on variegation, there may be some similarities in their effects on chromatin

structure. Of key interest here is whether suppressor genes can cause heterochromatic recombination as does butyrate. If the mutant has a general effect on recombination, male recombination might be observed even in the absence of P elements. On the other hand, if 325 acts in a manner analogous to butyrate, one might expect similar alterations in recombination pattern or frequency only in dysgenic crosses.

When Oregon R males are crossed to M females bearing the multiply marked second chromosome and Su(var)325 on the third chromosome (see Figure 2(b) for a diagram of this cross), no recombinants were observed in the 7,576 F2 progeny examined (data not shown). Thus, whatever effect 325 has on recombination, it does not promote either meiotic or somatic exchange in males.

The effects of introducing 325 into the dysgenic backgrounds of T-007 and OK1 are shown in Table 3. The resulting levels and distribution patterns of recombination in T-007 dysgenic males bearing 325 are not significantly different from those of the T-007 control (standard) experiment. Furthermore, the percent of clusters observed is not significantly altered (37 clustered events of 125 observed in total, or 30%).

Similarly, when 325 is introduced into the OK1 dysgenic cross, the effects on MR are only slight. For example, the only significant difference in regional recombination

P Strains		Genetic Interval					Total	Flies Scored
		1	2	3	4	5		
T-007	number[a]	31 (40)	38 (52)	0	38 (58)	18 (31)	125 (181)	9,921
	per cent[a]	.312 (.403)	.383 (.524)	0	.383 (.585)	.181 (.313)	1.26 (1.82)	
	distrib-tion[b]	.248	.304	0	.304	.144	1.000	
OK1	number[a]	35 (58)	68 (106)	0 0	85 (141)	24 (33)	212 (338)	22,448
	per cent[a]	.156 (.258)	.303 (.472)	0	.379 (.628)	.107 (.147)	.944 (1.51)	
	distri-bution[b]	.165	.321	0	.401	.113	1.000	

Table 3.   Recombination data, in each genetic interval, for dysgenic crosses involving P strains T-007 and OK1, to which the mutant Su(var)325 had been introduced.

a   data for recombination events given first, with total recombinants given in parentheses (see "Materials and Methods").

b   data based on recombination events only.

frequency that was observed for experimental vs. control males, was that for interval 2. Here again, clustering is not affected by the presence of 325. Seventy-one of the 212 events observed were clustered, which represents 34% of the events. Taken together, these results clearly indicate that the 325 mutation hsa virtually no effect on male recombination. Most significantly, it induces no heterochromatic recombination.

(d) Su(var)318

Preliminary mapping studies gave no indication that the mutant Su(var)318 influences meiotic crossing over. Itthus provides a contrast with the 325 mutation. As was true for 325, Su(var)318 failed to produce any male recombination among the 7,358 F2 progeny of the Oregon R control cross (data not shown). The results of the experiments to test the effects of 318 on male recombination in the dysgenic backgrounds of T-007 and OK1 are presented in Table 4. It can be seen from the data that 318 did not significantly alter recombination in the T-007 (13,662 flies scored) or OK1 (18,676 flies scored) experiments. For T-007 48 of the 155 observed recombination events were clustered (31%). For OK1, 50 of 151 events observed were clustered (33%). Neither of these values represents any significant difference from control levels.

P Strains		Genetic Interval 1	2	3	4	5	Total	Flies Scored
T-007	number[a]	35 (49)	43 (86)	0	49 (86)	29 (40)	155 (261)	13,662
	per cent[a]	.256 (.359)	.315 (.630)	0	.359 (.630)	.212 (.293)	1.14 (1.91)	
	distrib-tion[b]	.226	.277	0	.316	.187	1.000	
OK1	number[a]	23 (48)	42 (58)	0 0	63 (137)	23 (27)	151 (270)	18,676
	per cent[a]	.123 (.257)	.225 (.311)	0	.335 (.734)	.123 (.734)	.809 (1.45)	
	distri-bution[b]	.152	.278	0	.417	.152	1.000	

Table 4. Recombination data, in each genetic interval, for dysgenic crosses involving P strains T-007 and OK1, to which the mutant Su(var)325 had been introduced.

a data for recombination events given first, with total recombinants given in parentheses (see "Materials and Methods").

b data based on recombination events only.

Figures 3 and 4 (T-007 and OK1, respectively) show the second chromosome maps derived from data on the proportional distribution of recombination events for control dysgenic crosses and the butyrate and suppressor treatments. A genetic map derived from female meiotic recombination data is provided in each figure, for purposes of comparison. An asterisk indicates any region which shows a significant difference from the control dysgenic cross. In each figure, it can be seen that there is very little difference between the control cross and either suppressor cross.

Thus, these experiments have revealed no Su(var) effects on heterochromatic MR that are analogous to those of butyrate.
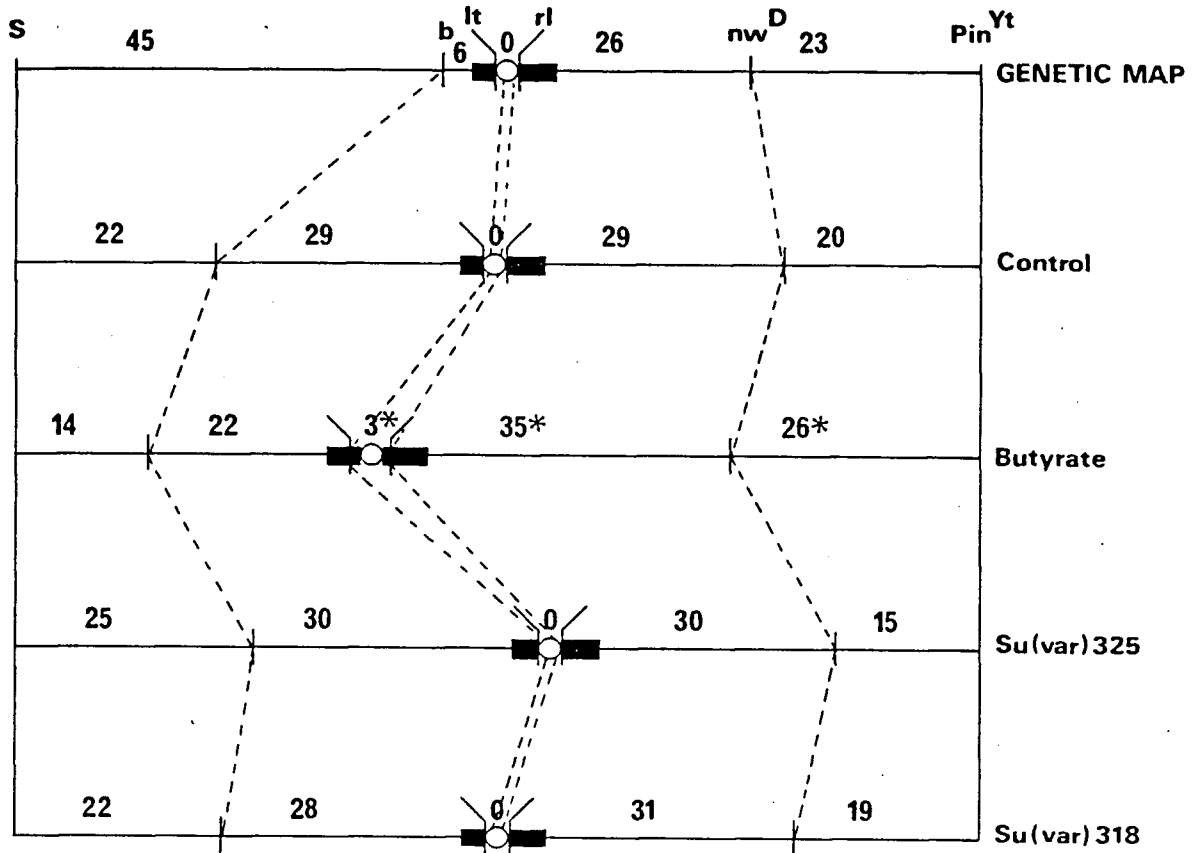
FIGURE 3. Second chromosome maps derived from recombination data of dysgenic crosses involving the P strain T-007. The upper line is derived from female meiotic recombination data, and is included for comparison. An asterisk indicates intervals which experience levels of recombination significantly different from controls (second line).
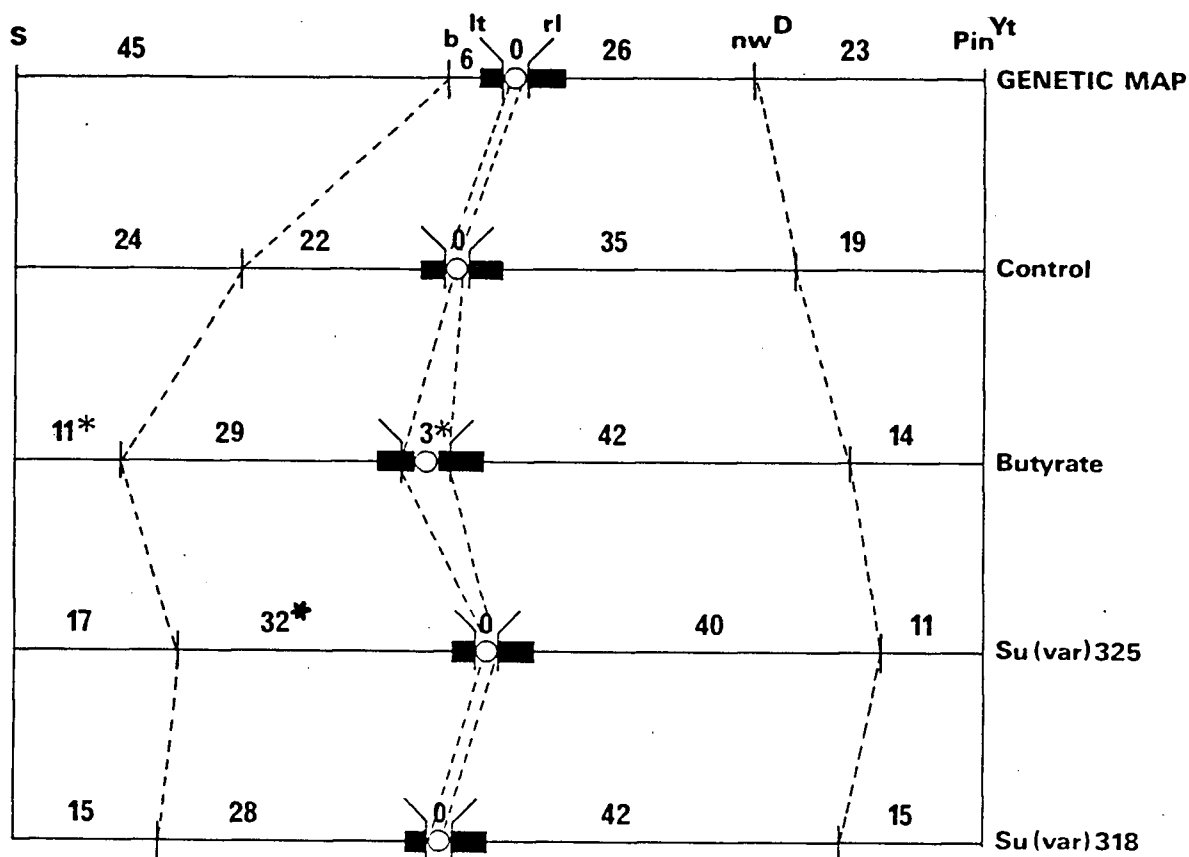
FIGURE 4. Second chromosome maps derived from recombination data of dysgenic crosses involving the P strain, OK1. The upper line is derived from female meiotic recombination data, and is included for comparison. An asterisk indicates intervals which experience levels of recombination significantly different from controls (second line).

DISCUSSION

Sinclair and Grigliatti (1985) reported that the T-007
and OK1 second chromosomes induce a characteristic frequency
and pattern of recombination. They found a non-random, strain
specific distribution of exchange along the second chromosome
for these P strains. The data presented in this report
confirm their results. The patterns generated by the control
crosses are very similar to those of Sinclair and Grigliatti,
especially that of OK1 which is nearly identical.

It has been shown previously (Hiraizumi et al., 1973;
Woodruff and Thompson, 1977), that most of the exchange
induced by the P strains T-007 and OK1 occurred within the
proximal intervals of the second chromosome. It was assumed
that this exchange took place in and around the centric
heterochromatin. Sinclari and Grigliatti (1985) and this
report show that this is not the case. Most, if not all, of
the exchange in these intervlas is euchromatic. Combining the
data from both studies: 15,639 chromosomes for T-007 and
23,399 for OK1 have been examined to date with no evidence of
heterochromatic exchange within the entirely heterochromatic
centromeric segment delineated by light and rolled. Since
both light and rolled are located within the heterochromatin
of the left and right arms of chromosome 2, it is possible

that recombination could have occurred in the segment of heterochromatin distal to each of these markers. While it is formally possible, it is not clear why recombination would occur in this portion of the centric heterochromatin but not within the region of heterochromatin flanked by lt and rl. In summary then, in both of these strains, heterochromatic crossing over if it occurs at all is extremely rare.

The simplest explanation for the lack of heterochromatic exchange events is that P elements do not normally insert into and/or subsquently excise from, the heterochromatic segments of a chromosome. This is supported by a number of studies in which the P element carrying a functioning gene was transformed into the Drosophila embryo (Goldberg et al., 1983; Richard et al., 1983; Scholnick et al., 1983; Spradling and Rubin, 1983; Hazelrigg et al., 1984). These P elements readily insert into the genome but rarely, if ever, do they insert into heterochromatin. In over 200 insertions identified to date only 2 elements exhibited a variegation effect, suggesting that they may have inserted into or near heterochromatin (Spradling and Rubin, 1983; and Hazelrigg et al., 1984). However, one of the elements inserted at the tip of the chromosome, possibly near the telomere. The other inserts at the euchromatin/heterochromatin junction and it is not possible to tell if it inserted into or simply near the heterochromatin.

Why would P elements be absent from heterochromatin? There are at least two possible explanations. Heterochromatin may lack the 8 bp recognition sequence into which the P elements insert. This recognition sequence is composed mostly of the bases G and C whereas heterochromatin is known to be very A-T rich. On the other hand, the recognition sequence is not highly conserved in composition, and considering that heterochromatin makes up approximately one fifth of the total DNA in a cell, it is difficult to imagine that some form of the recognition sequence is not present in heterochromatin. Another possibility is that an acceptable recognition sequence does exist in the heterochromatic regions of the Drosophila genome, but is normally inaccessible to P element insertion due to steric constraints imposed by the highly condensed state of the DNA. Transposable elements much larger than the relatively small P element are found in heterochromatin (Foldback and copia elements, for example; Truett et al., 1981, Ruddell, personal communication; for review see Rubin, 1983). If one hypothesizes that P elements are barred from the heterochromatic segments for steric reasons, then it is necessary to postulate further that the conditions and/or limitations on insertion of P elements must differ from those of those of the larger elements which are not similarly hampered. There is no a priori reason why all eucharyotic

elements should operate by the same "rules"; the various classes of prokaryotic transposable elements occasionally differ quite radically in their behaviour.

Butyrate was used to disrupt chromatin structure in an attempt to learn whether or not it is possible to reduce the "steric restraints" of highly condensed heterochromatin and allow P-induced recombination to take place. The observation that butyrate induced no recombination in the Oregon R cross is important because some chemicals are known to induce male recombination in the absence of P elements (Ferres et al., 1984). For example, two intercalating agents, ethidium bromide and acridine orange, cause significant levels of recombination on the third chromosomes of Drosophila melanogaster males. Presumably these intercalating agents disrupt the DNA helix to the extent that chromosome breakage occurs which is occasionally resolved via a homologous recombination event. However, ethoxy caffeine, another intercalating agent, fails to produce any male recombination. Ferres et al. suggest that ethidium bromide and acridine orange somehow suppress normal repair mechanisms so that broken strands are occasionally reannealed incorrectly to yield recombinant chromosomes. The alteration in chromatin structure induced by butyric acid is clearly a less direct effect than that of the above agents, or perhaps butyrate is unable to suppress the normal repair mechanisms. In any case,

the important point is that butyrate fails to induce male recombination in a non-dysgenic cross.

In contrast to the above lack of effect of butyrate on the previous crosses, butyrate does influence male recombination in the dysgenic crosses. It appears that butyrate can only affect male recombination in conjunction with the regular dysgenic machinery. The simplest conclusion that can be made is that butyrate facilitates insertion or excision of the elements by reducing the constraints imposed upon them by chromatin condensation.

The most important observation regarding the butyrate-treated dysgenic crosses is that for both P strains, a significant level of heterochromatic exchange was produced. This strongly favours the hypothesis that recognition sequences may be found in heterochromatin but are normally inaccessible to the elements. All of the heterochromatic exchanges observed were single (non-clustered) events. Whether this reflects a difference in timing of the exchange event in heterochromatin relative to euchromatin or in the nature of the exchange itself (if it were an imprecise exchange, for example, one would expect the "reciprocal" product to be lethal), or merely the result of a small sample size is not clear. Of the 11 events which were observed, about 32% of them, or four events, could have been expected to

be clusters (as noted in the results). Further crosses to increase the sample size may indicate whether or not the lack of clustering of the heterochromatic exchanges is significant. Sinclair and Grigliatti (1985) observed some heterochromatic exchange in a cross involving the P strain h12. Seven events were observed, among approximately 21,000 chromosomes examined, and each of these was also a single, unclustered event.

It is quite interesting to note that in the above study the strain h12 induced some heterochromatic exchange. why would this strain and none of the others tested display heterochromatic MR? Sinclair and Grigliatti suggest that P elements are present in the heterochromatin of this strain. There is another possible explanation. hybrid dysgenesis is known to have mobilized copia elements (Rubin et al., 1982). Copia elements and other members of the copia-like family show decided preferences for locations in heterochromatin (Finnegan, 1984; also Ruddell, personal communication). I suggest the possibility that h12 normally carries a quiescent copia-like element (perhaps gypsy) in the heterochromatin, near the lt locus, and that this element was mobilized during the dysgenic crosses to produce some recombination and chromosome rearrangements (which might be the basis for the lt mutations observed for this strain). This suggestion could easily be tested by in situ hybridization.

It is interesting that the two P strains responded
differently to butyrate treatment. The T-007 strain shows an
increased level of recombination in all three chromosomal
intervals examined on the right arm of chromosome two
(including the centromeric region). It may be that some
regions of euchromatin are more highly condensed than others;
in fact active chromatin is known to have a more "open"
conformation than inactive (as determined, for example, by
nuclease sensitivity studies), and perhaps butyrate relaxes
these regions and permits insertion and/or excision of P
elements with concommittant recombination in these regions.
Steric constraints, in other words, may be imposed by certain
regions of euchromatin as well as heterochromatin, and
defining such regions might prove quite interesting.

The OK1 strain showed very little change in pattern of
recombination (aside from interval number 3) as a result of
butyrate treatment and the overall frequency of recombination
was similar to that of the control. Why should the two
strains react so differently to butyrate treatment? Possibly
there exists some sequence polymorphism between them such that
when the more condensed euchromatic regions of the OK1 strain
are opened up by butyrate treatment there are no recognition
sequences newly exposed to permit element insertion into these
regions. Although it is likely that the two strains possess

some sequence differences (as they are geographically and temporally distinct), invoking sequence polymorphism may be unnecessary. A simpler explanation exists. The difference in response may simply reflect differing sites of residence of P elements in the two strains; the elements are known to reside in different locations among the various P strains examined (see Bregliano and Kidwell, 1983). Another possibility is that male recombination results from mobilization of elements other than P, such as copia elements, which are also present at different sites in the two P strains. It is known that hybrid dysgenesis causes mobilization of other elements (for example, the element mdg4; Gerasimova et al., 1984); whether the mobilization of these elements is associated with crossover is not known. In any case it is impossible to distinguish whether events observed here are the result of mobilization of P elements or other transposons.

Su(var)325 resides at position 53.3 ($\pm$1.4 map units) on the left arm of chromosome 3. It lies in the interval between the markers Gl (glued) and Sb (stubble) which includes the centric heterochromatin. In the process of mapping the gene, Tejani and Kroitsch (1982, personal communication) found that 325 causes a significant (50%)increase in the meiotic recombination frequency in this interval. I chose to study the effect of Su(var)325 on male recombination because of this possible effect on meiotic recombination. In contrast to 325,

the other Su(var) mutation used in this study, 318, had no such effect on meiotic recombination. 318 lies in the same genetic interval as 325 at position 56.1. Both 325 and 318 are part of a cluster of Su(var) genes and may be structurally and/or functionally related (Sinclair et al., 1983).

When a cross is made between M strain females bearing the multiply marked second chromosome and a Su(var)325-bearing chromosome and M strain Oregon R males, there is no recombination observed in the resulting F1 progeny. This eliminates the possibility that 325 causes localized chromosome breakage and male recombination. If Su(var)325 alters male recombination frequencies at all, it appears to do so by augmenting the normal mechanisms of recombination--either meiotic or dysgenic--and not via some other independent process.

The presence of 325 in the dysgenic cross in which the original male parent is of the OK1 strain increased the overall amount of male recombination. The distribution of recombinant events was similar to that found in the dysgenic cross lacking 325, with the exception of the b to lt interval. In this interval (number 2), the percent recombination was nearly twice that of the standard dysgenic cross. This is comparable to the 1.5-fold increase in meiotic recombination frequency observed during the mapping of the gene on

chromosome three. 325 did not cause any changes in recombination frequency in the T-007 cross.

Su(var)318 had no effect on either P strain, which suggests that it may act quite differently from 325. If 318 and 325 are really part of a functionally related cluster of genes, their functions are sufficiently distinct to cause different responses in the dysgenic crosses. 318 may act upon some aspect of the chromatin structure right in the transition region, so specifically that recombination, both dysgenic and meiotic are unaffected. It apparently does not act upon heterochromatin, or does so in a manner which does not allow dysgenic recombination to take place in the interval between lt and rl. This is as expected given the effect of 325 and 318 on meiotic recombination. 325 alters meiotic recombination as well as dysgenic, while 318 alters neither. Previous studies have shown (see Engels, 1983) that the factors which are known to alter meiotic recombination do not seem to affect male recombination. The mutant 325 is apparently an exception to this observation as it seems to affect both types of recombination in an analogous manner.

325, then, acts on analagous areas: the left hand euchromatin/heterochromatin boundary of chromosome two (MR) and the right of left hand boundary of chromosome three (meiotic recombination in females), to increase recombination frequency. It also acts on the X chromosome to suppress the

inactivation of $\underline{w}^+$ in $\underline{w}^{m4}$, and it is likely that it acts on the euchromatin/heterochromatin boundary of this chromosome also.

The euchromatin/heterochromatin boundary is a region of gradual rather than abrupt change (Lifschytz, 1978). It appears cytologically as a rather diffuse stretch of "B" heterochromatin, gradually changing to the more highly condensed "A" (centric) heterochromatin (Lakhotia and Jacob, 1974). In the transition zone there are euchromatic sequences interspersed between blocks of intercalary heterochromatin. This apparently makes up the structure of the less diffuse B-type heterochromatin.

If, as seems likely, suppressor genes are involved in establishing, maintaining or regulating chromatin structure, then it is logical to look for similarities in the makeup of the chromatin in those regions which appear to be affected by the 325 mutant: the left euchromatin/heterochromatin boundary of chromosome two, the left and/or right (because both are included in the interval between Gl and Sb) boundary of chromosome three, and the transition zone of the X chromosome. It may be possible in this way to pinpoint the aspect of chromatin structure upon which 325 has its effect.

About 80% of heterochromatin is composed of satellite sequences: short highly repeated sequences occupying distinct

regions on individual chromosomes. There are six major satellite sequences in <u>Drosophila</u> <u>melanogaster</u>, each distinguishable on the basis of its sedimentation in cesium chloride density gradients. A satellite sequence is an obvious aspect of chromosome makeup which can be easily examined for presence or absence in the areas of interest. Interactions between euchromatic genes or gene products and heterochromatic sequencs are not unknown. For example, there are certain proteins known to be preferentially associated with certain satellite sequences (Hsieh and Brutlag, 1979; Levinger and Varshavsky, 1982). In addition, Sandler (1977) observed that two maternal effect mutants of <u>D.</u> <u>melanogaster</u>, <u>abo</u> (abnormal oocyte) and <u>da</u> (daughterless), could be partially rescued in the presence of extra copies of certain X heterochromatic sequences. Each gene interacted with one specific sequence. I envision the possibility of an analogous relationship between the <u>325</u> mutant and a satellite sequence. The <u>325</u> product may interact with this sequence to cause loosening of the DNA structure such that recombination is enhanced or suppression of variegation is possible. There is one sequence -1.697g/cc- which is found on the X heterochro-matin and left transition boundaries of chromosomes two and three (Peacock <u>et al.</u>, 1973). However, it is also found on the right boundary of chromosome two, where <u>325</u> appears to have no effect. This may be explained in one of two ways:

either 325 is not in any way involved with this particular satellite sequence, and its presence in the regions of interest is coincidental; or it does interact with the 1.697 sequence, but no dysgenic recombination is observed on the right boundary of chromosome two due to the paucity of appropriate recognition sites--or P elements--in that region. Given the highly conserved sequence structure of satellites, it seems unlikely that recognition sequences present in the left hand block of 1.697 would not also be present in the right hand block. There is some evidence, also, that the suppressor gene acts on euchromatic rather than heterochromatic sequences (see later discussion).

The effect of the 325 mutation may be very straightforward. It may act upon any heterochromatin/euchromatin boundary with the net effect of releasing some sequences from the tightly compressed regions of the transition zone to a less condensed form. The effect, or lack of one, on dysgenic recombination may then stem directly from the presence or absence of P-element recognition sites in the areas which have been newly exposed. These newly exposed regions need not be satellite sequences, as unique sequences, as well as blocks of euchromatin, are known to reside in the transition zone. These sequences will not be the same for any two transition regions, and can thus explain the differences

between the responses of various transition regions in the presence of 325.

If the suppression activity of 325 operates by the same mechanism as does the effect on recombination--which is the simplest possibility--then the suppressor gene may not affect heterochromatin at all, contrary to initial expectations (see Introduction). Rather, the mutant may confer upon euchromatin the ability to resist heterochromatic spreading. Two observations suggest that the mutant 325 may affect euchromatin rather than heterochromatin. No heterochromatic crossing over was observed in either dysgenic cross in the presence of 325. 325 is a stronger suppressor of variegation than is butyrate, yet it did not affect the heterochromatin as did butyrate. Thus, the mutant must act on a different component of chromosome structure than does butyrate, and probably more specifically. Therefore, 325 probably acts upon the euchromatin in and around the transition zone (the only region where it appears to have any effect at all). In addition, the fact that meiotic recombination is altered by 325 indicates that 325 most likely acts upon euchromatic sequences, since meiotic recombination is very rare in heterochromatin, and rather infrequent in the region of the transition zone.

CONCLUSIONS

In dysgenic crosses involving the P strains T-007 and OK1, rarely, if ever, does heterochromatic exchange occur on chromosome two. It was postulated that this lack of exchange reflected steric constraints due to the tight packaging of heterochromatin. This hypothesis was tested by exposing dysgenic hybrid males from either strain to .15M butyric acid, a presumed modifier of chromatin structure. In both P strains, the butyric acid induced significant levels of male recombination in the heterochromatic lt to rl interval of chromosome two. Thus it appears that chromatin structure can influence the insertion and/or excision of the elements and this is reflected in male recombination. Differences in response to butyrate by the two P strains may reflect differences in the sites of residence of P elements in each strain.

The same dysgenic crosses were performed in combination with two Su(var) genes: 325 and 318. Neither produced any heterochromatic recombination, which suggests that their modes of action may be quite different from, and more specific than, the rather general effects of butyrate.

325 caused an increase in the recombination frequency in the interval containing the left heterochromatin/euchromatin

boundary of chromosome two of the OK1 strain. It is suggested that the 325 mutant most likely interacts with the euchromatin (although the possibility of an interaction with heterochromatin is also discussed) in the transition zone, and acts to release some sequences from their highly condensed state to a less compressed form. In areas where newly exposed sequences contain either recognition sequences for P elements, or the elements themselves, dysgenic recombination may take place. The fact that the right boundary of chromosome two does not act similarly and that the T-007 strain experiences no similar increase in male recombination may be explained by differing sites of residence of the P elements in different chromosome locations, and between strains.

The most important observation is that altering chromatin structure does not appear to influence dysgenic recombination and thus (by inference) the mobility of the P elements. At present, techniques exist by which P elements may be introduced into the germ line of Drosophila embryos, but it is not yet possible to direct the insertion of the elements, usually carriers for functioning copies of genes which can "rescue" individuals lacking functional copies of the gene. The recognition sequence, or a recognition-like sequence is one prerequisite for the direction of a P element to a particular insertion site; the state of the chromatin at that site is apparently another.

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APPENDIX I

I performed an additional experiment with the P strain OK1. A reciprocal cross (one in which the maternal parent was of the P strain, and the paternal parent was the M strain bearing the multiply marked second chromosome) was set up and treated with 0.15M butyrate, as outlined in the MATERIALS AND METHODS section. This was done to ascertain that butyrate did not act outside of the regular dysgenic mechanisms (to derepress the P elements, for example, despite their being in a P cytotype). 10,183 F2 progeny were scored, and 13 recombinant events were observed (36 recombinant individuals), 5 (7 recombinant individuals) in interval 2 and 8 (29 individuals) in interval 4. There was no recombination observed in any other interval.

A control (the reciprocal cross, not treated with butyrate) was not performed, but analogous experiments have been performed numerous times (see Bregliano and Kidwell, 1983, for review). These studies suggest that the reciprocal cross should yield male recombination at frequencies about ten-fold lower than those of the dysgenic cross. If the reciprocal cross had been performed and had yielded frequencies fifteen times lower than the dysgenic cross, there would have been no significant (G test, $p = .05$) difference

between this cross and the reciprocal cross treated with butyrate. Thus, butyrate appears not to operate outside of the dysgenic system. Most important, it cause no heterochromatic recombination outside of the dysgenic system. However, because the control (untreated reciprocal) cross was not actually performed, I decided to place this observation in an appendix, rather than in the text.