Cis-Effects of Heterochromatin on Heterochromatic and Euchromatic Gene Activity in *Drosophila melanogaster*

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ABSTRACT

Chromosomal rearrangements that juxtapose heterochromatin and euchromatin can result in mosaic inactivation of heterochromatic and euchromatic genes. This phenomenon, position effect variegation (PEV), suggests that heterochromatic and euchromatic genes differ in their regulatory requirements. This report describes a novel method for mapping regions required for heterochromatic genes, and those that induce PEV of a euchromatic gene. *P* transposase mutagenesis was used to generate derivatives of a translocation that variegated for the *light*$^+$ (*lt*$^+$) gene and carried the euchromatic *white*$^+$ (*w*$^+$) gene on a transposon near the heterochromatin-euchromatin junction. Cytogenetic and genetic analyses of the derivatives showed that *P* mutagenesis resulted in deletions of several megabases of heterochromatin. Genetic and molecular studies showed that the derivatives shared a euchromatic breakpoint but differed in their heterochromatic breakpoint and their effects on seven heterochromatic genes and the *w*$^+$ gene. Heterochromatic genes differed in their response to deletions. The *lt*$^+$ gene was sensitive to the amount of heterochromatin at the breakpoint but the heterochromatic *40Fa* gene was not. The severity of variegated *w*$^+$ phenotype did not depend on the amount of heterochromatin in *cis*, but varied with local heterochromatic environment. These data are relevant for considering mechanisms of PEV of both heterochromatic and euchromatic genes.

ONE of the earliest observations on chromosome organization was the identification of two cytologically distinct domains, euchromatin and heterochromatin. HEITZ (1928) defined euchromatin as regions that condense during mitosis and appear decondensed in interphase. Heterochromatin was defined as regions that remain visibly condensed throughout the mitotic cell cycle, including interphase. Although this first description of heterochromatin was morphological, subsequent work has shown that heterochromatic regions share other properties, including an abundance of repetitive DNA and reduced recombination rates (reviewed by JOHN 1988).

Extensive evidence that heterochromatin and euchromatin are functionally different domains in terms of gene expression, comes from studies of position dependent gene inactivation or position effect variegation (PEV) in Drosophila. When a gene is moved from its normal location in one domain to a site within the other domain, it is often subject to mosaic inactivation. The most familiar example of PEV is the heterochromatin-induced inactivation of the euchromatic *white*+ (*w*$^+$) gene, which results in a mosaic red and white eye phenotype. A wide variety of euchromatic genes show PEV when moved near the heterochromatin by chromosomal rearrangements or when inserted into the heterochromatin by *P* element transposition. These variegating euchromatic genes are influenced in similar ways by modifiers of PEV that include the addition of the heterochromatic *Y* chromosome and mutations known as suppressors and enhancers of variegation (reviewed by GRIGLIATTI 1991).

While the vast majority of identified genes in Drosophila are euchromatic, >30 genetic functions reside within heterochromatin (reviewed by GATTI and PIMPINELLI 1992). SCHULTZ (1936) and HESSLER (1958) were the first to demonstrate that a heterochromatic gene, the *light*$^+$ (*lt*$^+$) gene, shows PEV when displaced from centromeric heterochromatin to distal euchromatic regions. Subsequent studies have shown that proximity to heterochromatin is a general requirement for normal levels of expression of six heterochromatic genes, in addition to the *lt*$^+$ gene (WAKIMOTO and HEARN 1990; EBERL *et al.* 1993). Variegating heterochromatic genes can also be affected by the *Y* chromosome or by genic modifiers of PEV. However, the effect is opposite to that seen with variegating euchromatic genes. That is, the addition of extra heterochromatin (SCHULTZ 1936; BAKER and REIN 1962) or mutations that suppress PEV of euchromatic genes enhance the variegation of heterochromatic genes (HEARN *et al.* 1991). These data have led to the conclusion that regulatory requirements for at least a subset of heterochromatic genes must differ in some ways from those of euchromatic genes (WAKIMOTO and HEARN 1990; EBERL *et al.* 1993).

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An understanding of the molecular mechanisms underlying PEV and the regulatory differences between euchromatic and heterochromatic genes requires that we understand why some chromosomal rearrangements evoke strong position effects on genes while others have weak or no effects. Previous studies have attempted to address this question by comparing the properties of different rearrangements that variegate for a particular gene. The strength of position effects has been attributed to a variety of factors. The position of the variegating allele along the chromosome arm and its position relative to other regions of constitutive heterochromatin are important determinants influencing the strength of variegation of heterochromatic genes (HESSLER 1958; WAKIMOTO and HEARN 1990; EBERL *et al.* 1993). These factors have recently been shown to affect a variegating euchromatic gene as well (TALBERT *et al.* 1994). The source of heterochromatin at the breakpoint is also a major factor. Studies by SPOFFORD (1976) and SPOFFORD and DESALLE (1991) demonstrate that different regions of heterochromatin are not equally effective at inducing PEV on euchromatic genes. TARTOF *et al.* (1984) suggested that specific sequences are capable of either initiating heterochromatin formation or terminating the spread of heterochromatin into euchromatin. According to their model, the strength of the position effect is influenced by proximity of the affected gene to the proposed heterochromatin initiator or terminator sequences. In contrast, REUTER *et al.* (1985) proposed that position effects differ in strength because of variation in number of repetitive sequences adjacent to the heterochromatin-euchromatin junction, as opposed to specific initiators or terminators.

A critical evaluation of the contributions of factors affecting PEV has been difficult for several reasons. The rearrangements being compared in previous studies often differed in multiple parameters including the complexity of the rearrangements, differences in the genetic background of the strains used and the location of the euchromatic breakpoint. Additional variations included the source and the size of the heterochromatic block at the breakpoint. Only a few studies (TARTOF *et al.* 1984; POKHOLKOVA *et al.* 1993) molecularly compared the heterochromatin at euchromatin-heterochromatin junction in different rearrangements. However, none of the earlier studies could critically assess whether variations in the type of heterochromatic sequence or the size of the heterochromatic block at the junction was responsible for differences in the strength of PEV.

This paper describes a scheme devised to overcome many of the difficulties encountered in previous studies. We have generated a series of overlapping deficiencies of the heterochromatin of the left arm of chromosome 2 (2Lh). The chromosomes were derived from the same parental chromosome. Genetic, cytogenetic and molecular characterizations of these chromosomes showed that they differed only in the amount of 2Lh at the variegating euchromatin-heterochromatin junction. Using these chromosomes, we directly compared the effects of specific regions of heterochromatin on the variegation of a euchromatic gene and seven heterochromatic genes. These chromosomes also provided the tools that allowed us to begin a molecular characterization of the distribution and types of sequences within 2Lh.

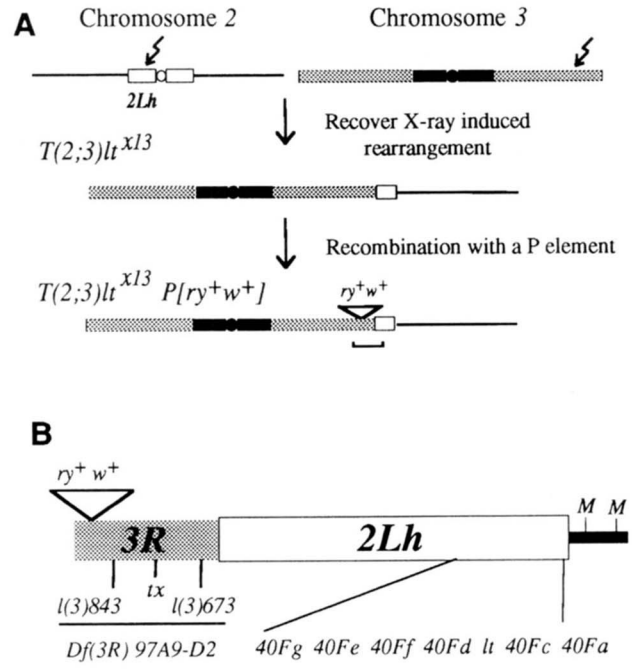

FIGURE 1.—Generation and structure of the *T(2;3) lt^{x13} P[ry$^+$ w$^+$]* chromosome. (A) The *T(2;3) lt^{x13}* chromosome was recovered as an X-ray induced reciprocal translocation by WAKIMOTO and HEARN (1990). Jagged arrows show the position of the breaks produced by X irradiation. The orientation of the *P* element introduced by recombination to produce *T(2;3) lt^{x13} P[ry$^+$ w$^+$]* was deduced from the molecular studies described in the text. (B) Expanded view of the euchromatin-heterochromatin junction bracketed in A. The proximal-distal order of the genetic complementation groups used to characterize the derivatives are shown in the 3R euchromatin (stippled box), in 2Lh (white box) and in 2L euchromatin (thick line).

MATERIALS AND METHODS

Drosophila stocks and chromosomes: Cultures were raised at 25° unless noted otherwise on cornmeal-agar-molasses-Brewer's yeast media with Tegosept added as a mold inhibitor. Strains carrying lesions in *97C* were generously provided by K. V. ANDERSON. The *P[(w, ry)F] 4–3* strain was constructed by LEVIS *et al.* (1985) and obtained from the Bloomington Stock Center. The *lt^1* and *tx^1* mutations are homozygous viable, hypomorphic alleles of the *light* and *taxi* genes respectively. Other mutations and chromosomes are described in LINDSLEY and ZIMM (1992), WAKIMOTO and HEARN (1990), or in the text below.

Figure 1A summarizes the scheme used to generate the chromosomes used in this study. The *lt*-variegating translocation *T(2;3) lt^{x13}* is a reciprocal translocation with breakpoints in 2Lh and *97C* (WAKIMOTO and HEARN 1990). The dominant *Sternopleural* (*Sp*) mutation and a marked *P* element were introduced onto the translocation by recombination. For the *P* element recombination, flies carrying *T(2;3) Sp lt^{x13}* were

crossed to the *P*[(*w*, *ry*)F] 4–3 strain (LEVIS *et al.* 1985). The *P* element (hereafter denoted *P*[ry^+w^+]) is inserted into *97B* and carries the $rosy^+$ gene and a 8.2-kb *ScaI-KpnI* fragment of the $white^+$ gene. Females that were w^{1118};*T(2;3) Sp* lt^{x13}/*SM1, Cy* lt^H;*P*[ry^+ w^+] were mated to w^{1118}; lt^1 stw^3 males. Their progeny were scored for independent segregation of *Cy* and w^+ markers to recover the *T(2;3) Sp* lt^{x13} *P*[ry^+ w^+] recombinant class. The *P* element maps 0.09 map units proximal to the breakpoint of the translocation (31 recombinant chromosomes from 34,179 progeny). One recombinant translocation was established in a balanced stock as w^{1118};*T(2;3) Sp* lt^{x13} *P*[ry^+ w^+]/*SM1,Cy* lt^H;*TM6B, Hu Tb* and used for all subsequent experiments. The location of the *P* element in this strain was confirmed by *in situ* hybridization of a labeled ry^+ probe onto polytene chromosomes using the protocol of LIM (1992). Hybridization was observed adjacent to the 2Lh;*97C* heterochromatin-euchromatin junction. The orientation of the *P* element shown in Figure 1 was determined by molecular studies described in the text.

***P* transposase mutageneses:** Two screens were used to generate derivatives of *T(2;3) Sp* lt^{x13} *P*[ry^+ w^+]. In both screens, the translocation was exposed to a genomic source of *P* transposase (ROBERTSON *et al.* 1988) in males of the genotype w^{1118};*T(2;3) Sp* lt^{x13} *P*[ry^+ w^+]/lt^1 stw^3;*P*[$ry^+\Delta 2-3$]. For the first screen, these males were mated *en masse* to lt^1 stw^3 females at 18 or 25°. The progeny of this cross were scored for severe lt-variegating or lt^- eye color phenotypes. In the second scheme, single males of the genotype above were crossed to three to five females homozygous for the $taxi^1$ (tx^1) mutation in vials, and their progeny were scored for the taxi mutant heldout-wing phenotype by scanning the vials visually. The *T(2;3) Sp* lt^{x13} *P*[ry^+ w^+] chromosomes carrying new *lt* or *tx* lesions (designated *T(2;3)**) were recovered, then established in balanced stocks over *SM1* and *TM6B*, or over *SM1* alone.

Complementation analyses: The *T(2;3)** derivatives were tested for their ability to complement mutations that mapped to either side of the 2Lh;*97C* breakpoint (Figure 1B). The *97B-D* lesions included: the *Df(3R)Tl*^{84cRPA}, which deletes *97A9,10–97D2* (ANDERSON *et al.* 1985), the *l(3)834* and *l(3)673* mutations (K. V. ANDERSON, unpublished data). The EMS-induced lethal or semilethal mutations in the 2Lh genes were: *EMS 40–5, 56–24, 56–4, 40–7, 40–12, 40–2* and *56–8* that represent the *40Fg, 40Fe, 40Ff, 40Fd, lt, 40Fc* and the *40Fa* genes respectively (HILLIKER 1976; LINDSLEY and ZIMM 1992). For the complementation tests with the 2Lh mutations, three to five females heterozygous for one of the 2Lh mutations and either the *SM1* or *Gla* balancer were mated to *T(2;3)*/SM1;TM6B* males in vials. Three to seven vial crosses were scored for each of the combinations reported in Table 3, except for those involving tx^{p5}, which were performed only once. For all of the combinations, a minimum of 150 but generally >300 progeny were scored. The viability of the critical *T(2;3)*/EMS* lethal class was expressed relative to that of the *T(2;3)*/Balancer* sibs. This sibling class repeatedly showed the greatest viability of all four progeny classes and was therefore chosen as the most rigorous control class. Ratios of the classes were determined for each separate cross of pairwise genotypes. These ratios were analyzed by analysis of variance (ANOVA) with the SuperANOVA software package. *P* values from the sum of squares are reported.

Assays for *lt*-dependent Malpighian tubule pigmentation: Malpighian tubules from third instar male larvae of the genotype *T(2;3)*/*lt^1 were dissected in Ephrussi-Beadle Ringers (ASHBURNER 1989), immediately transferred to a solution of 1 μg/ml of the DNA stain DAPI and mounted on multiple well depression slides. Tubules were inspected using UV epifluorescent optics at ×100 magnification on a Nikon Microphot FX. Thirty cells per tubule arm and two arms per individual were scored for the presence or absence of large, autofluorescent pigment granules. Fully and intermediately pigmented cells were scored as pigmented. The hypomorphic lt^1 mutation results in cells with small homogeneous granules and these cells were scored as unpigmented. At least 15 individuals per genotype were scored.

Cytology: To analyze the structure of the *T(2;3)*lt^{x13} translocation, chromosome squashes were prepared from larvae homozygous for the translocation. The *T(2;3)* lt^{x13} *P*[ry^+ w^+] chromosome and its derivatives were examined in heterozygous combination with *T(2;3)*lt^{x13} because these combinations were viable. Polytene chromosome squashes were stained with lactoaceto-orcein as described by ASHBURNER (1989) and analyzed by phase contrast microscopy. Neuroblast chromosomes were stained for Hoeschts 33258-, DAPI- and N-banding as described by PIMPINELLI and DIMITRI (1989). Hoescht and DAPI staining reveal similar banding patterns, with preferential staining of A-T rich heterochromatic regions. Mitotic chromosome preparations and fluorescent *in situ* hybridization (FISH) were carried out as described by GATTI *et al.* (1994). The probes and their precise positions on the mitotic chromosome map were described by PIMPINELLI *et al.* (1995). The 1.705 satellite sequences were detected using a probe that recognizes the AAGAG and AAGAGAG repeats (BONACCORSI and LOHE 1991; LOHE *et al.* 1993). The *Doc* retrotransposon was detected by labeling sequences in the pDocO'Hare plasmid provided by K. O'HARE (unpublished data).

Southern blot analyses: Genomic DNA was isolated according to BENDER et al. (1983) for small scale preparations or by CsCl gradient centrifugation for large scale preparations. Restriction digests, gel electrophoresis, and Southern blotting to Hybond-N membranes (Amersham, Arlington Heights, IL) were performed essentially as described by SAMBROOK *et al.* (1989). Probes were labeled with ^{32}P-dCTP using the Prime-it II kit according to the manufacturer's recommendation (Stratagene, La Jolla, CA). The probe used to detect *light* gene sequences was the pltB1.7 plasmid, which contains a 1.7-kb *Hin*dIII-*Hin*dII fragment of the lt^+ gene (B. WAKIMOTO, unpublished data). A 550-bp *Hin*dIII-*Eco*RI fragment from the Carnegie 2 plasmid (RUBIN and SPRADLING 1983) was used to detect the 5′ *P* element end. An 8-kb *Sal*I fragment containing the ry^+ gene (LEE *et al.* 1987) and the 1.3-kb *Kpn*I- *Sal*I fragment at the 3′ end of the $white^+$ gene (LEVIS *et al.* 1982) were used to detect sequences within or flanking the *P*-element transposon.

DNA amplification: Genomic DNA was amplified using the inverse polymerase chain reaction (PCR) described by OCHMAN *et al.* (1990). DNA from ~25 flies was digested with *Kpn*I. Subsequent digest with another restriction enzyme, followed by treatment with the Klenow fragment of *Escherichia coli* DNA polymerase was used to generate blunt ends when necessary to generate a suitably small PCR target template. One-tenth of the DNA was used in a 50-μl ligation reaction overnight. The ligated DNA was amplified using primers 5P.21 (5′-ACGTGACTGTGCGTTAGGTCC-3′) and IRXb (5′-GCCCATCGGGACCACCTTATGTTA-3′). The primers recognize sequences within the 5′ *P* element end and were generously provided by C. BERG. One-tenth of the ligation reaction was amplified in a 100 ul PCR reaction using *Taq* Polymerase according to the conditions specified by the enzyme supplier (Promega, Madison, WI). Gel purified PCR products were radiolabeled, then used as probes for Southern blots.

RESULTS

Experimental rationale: Our aim was to generate a series of deletions of the heterochromatin of the left arm of chromosome 2 (2Lh), to compare the effects of specific blocks of heterochromatin on the expression

of genes in 2Lh. We induced these deletions on a reciprocal translocation by *P* transposase mutagenesis. The starting material was a translocation, *T(2;3) lt*^{x13}, which was isolated as an X-ray induced lesion that variegates for the heterochromatic *light*$^{+}$ gene (WAKIMOTO and HEARN 1990). To direct *P* transposase activity to the displaced 2Lh, we introduced a marked *P* element to a position just proximal to the euchromatic-heterochromatic breakpoint. Figure 1 summarizes the strategy used to generate the resulting translocation, designated *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$], and the structure of the heterochromatin-euchromatin junction.

The *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] chromosome has three advantages for the genetic and physical mapping of 2Lh. First, the breakpoint isolates approximately half of 2Lh and seven essential heterochromatic genes from a centromeric position to a distal euchromatic region (WAKIMOTO and HEARN 1990). Second, the translocation is homozygous viable, indicating that the displaced 2Lh contains the *cis*-acting elements necessary for the expression of the 2Lh genes. Third, the euchromatic breakpoint of *T(2;3) lt*^{x13} resides in a genetically well-characterized region, *97C*. The mutations in this region (ANDERSON *et al.* 1985; K. V. ANDERSON, unpublished results) and cloned sequences from *P* transposon provided useful tools for genetic and molecular characterizations.

Recovery of the *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] derivatives: Two screens were used to generate *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] derivatives that were deleted for 2Lh. The transposase source *P*[*ry*$^{+}\Delta$ 2–3] (*99B*) was used as the mutagen in both screens, with the hope that all of the deficiencies would share one breakpoint near the *P*[*ry*$^{+}$ *w*$^{+}$] element. The first screen was designed to recover deletions that remove sequences important for 2Lh gene expression. The second screen was designed to recover 2Lh deletions without selection for effects on heterochromatic gene expression.

In the first screen, dysgenic males carrying *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] and the *P*[*ry*$^{+}\Delta$ 2–3] (*99B*) source of transposase were mated to females homozygous for the hypomorphic *lt*^{1} allele. Their progeny were scored for a lt-variegated or lt^{-} phenotype that differed from the parental phenotype. From ~48,000 progeny, seven lines, representing at least six independent events, were established. These lines were tested for their ability to complement a deficiency in the *97A-D* interval (Figure 1B). Three lines failed to complement the 3R deficiency and were considered good candidates for deletions that extended into 2Lh.

The candidate 2Lh deletion derivatives recovered in the first screen failed to complement a mutation in *taxi* (*tx*), a gene that is located in *97B* (K. V. ANDERSON, unpublished results). This result indicated that a screen for *tx*$^{-}$ derivatives of *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] should permit the recovery of lesions near or at the heterochromatin-euchromatin junction, without imposing a selection for those that affected *lt*. We performed a second screen in which single dysgenic males which carried the *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] chromosome were mated to females homozygous for the *tx*^{1} mutation. Of the ~58,000 F_1 progeny scored, 39 *tx* mutants, representing ≥26 independent events, were recovered.

TABLE 1

Complementation patterns of Class I–V derivatives

	3R genes				2Lh	2L euc
Class	*Df(3R)*	*843*	*tx*	*673*	*lt*	*Minute (M)*
Parent	+	+	+	+	−	+
I	+	+	+	+	−	+
II	+	nd	nd	nd	−	+
III	nd	−	−	+	−	+
IV	−	−	−	−	−	+
V	−	−	−	−	−	−

Complementation analysis of the derivative define five classes. +, full complementation; nd, not determined; −, failure to complement.

Classification of the *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] derivatives: The derivatives of *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] that were most useful for our study were those that carried deletions that began at or near the *P* element and extended variable distances into 2Lh. To distinguish these heterochromatic deficiencies from the other classes of *lt*$^{-}$ or *tx*$^{-}$ mutations, we used complementation analysis to determine if the lesions affected one or more of the genes that mapped to the euchromatic *97A-D* interval, 2Lh or 2L euchromatin (Figure 1B).

Complementation analyses with the derivatives allowed us to divide them into five classes. The genetic criteria for this classification are summarized in Table 1. The number and origin of derivatives in each class are listed in Table 2. Class I was represented by a single chromosome that induced a more severe lt-variegated phenotype than the parental chromosome and complemented all the third chromosome mutations. The lesion associated with this chromosome could have been closely linked to the *lt* gene or might have mapped elsewhere, acting as a dominant modifier of *lt* expression or a modifier of PEV. Class II chromosomes, isolated from the first screen, appeared to have lesions restricted to the *lt* gene. They resulted in a lt^{-}, rather than a lt-variegated

TABLE 2

Summary of the results of the screens for *T(2;3)lt*^{x13}P**[*ry*$^{+}$*w*$^{+}$] derivatives**

		Number derivatives per class[a]				
Screen	*n*	I	II	III	IV	V
lt screen	48,000	1	2	0	2	1
tx screen	58,000		0	14	6	1
Total	106,000	1	2	14	8	2

[a] Criteria for classification is described in the text.

phenotype and complemented mutations in all of the other 3R and 2Lh genes tested. Class III chromosomes carried deletions limited to 3R euchromatin as they affected the *l(3)843* and *tx* genes, but not the *l(3)673* gene. Class IV and Class V chromosomes failed to complement the point mutations in each of the 3R lethal complementation groups [the *l(3)843, tx* and *l(3)673* genes] and mutations in one or more of the 2Lh genes. These derivatives were considered good candidates for deletions that extended into 2Lh.

The two Class V chromosomes, tx^{p17} and $lt^{x13p100}$, were associated with a strong dominant Minute phenotype. While $lt^{x13p100}$ was lost, tx^{p17} was established in a stock. The Minute phenotype associated with tx^{p17} was rescued by *Dp(Y;2) G*, a *Y* chromosome that carries an insertional translocation of regions *36B–40F* (LINDSLEY and ZIMM 1992). Two haplo-insufficient *Minute* loci map to the proximal euchromatin of 2L, one in region *39F* and the other in *40B-F* (S. KERRIDGE, personal communication; B. WAKIMOTO, unpublished observations); when both loci are deleted, a strong Minute phenotype is observed. These data indicated that at least one Class V chromosome was an extremely large deletion that extended from the $P[ry^+\ w^+]$ element in 3R to at least region *39F*, resulting in the removal of the entire block of translocated 2Lh and some adjacent euchromatin.

Class IV derivatives had differential effects on 2Lh genes: Our subsequent experiments concentrated on the eight Class IV chromosomes. Two of these were recovered in the screen for *lt* mutations and were designated lt^{x13p20} and lt^{x13p89}. Six chromosomes were recovered as tx^- mutations and were designated tx^{p1}, tx^{p5}, tx^{p15}, tx^{p18}, tx^{p19} and tx^{p31}. These chromosomes were not associated with a Minute phenotype indicating that the deficiencies might terminate in 2Lh. The results of the lethal complementation analysis are described below and summarized in Table 3.

The *T(2;3)* lt^{x13} $P[ry^+\ w^+]$ parental chromosome fully complemented mutations in four 2Lh genes and partially complemented mutations in three others. There is extreme variability in expression of the tester alleles for the two most proximal genes *40Fg* and *40Fe*. The tester alleles are semilethal and when hemizygous, they permit from 20–70% viability (WAKIMOTO and HEARN 1990). Only the tx^{p31} chromosome showed complementation behavior similar to the parental chromosome with a mutant allele of the most proximal 2Lh gene, *40Fg*. The simplest interpretation of this result was that the *40Fg* gene and the 2Lh genes distal to it were present on the tx^{p31} chromosome. The remaining derivatives differed from the parental chromosome in their complementation behavior with the *40Fg* mutation. It was unclear if this gene was deleted from all or some of these chromosomes or if this pattern resulted from position effects on the gene. Either explanation might also have accounted for the failure of the derivatives to complement mutations in the *40Fe* and *40Ff* genes. However, based on the argument above, it is likely that these genes were retained on tx^{p31} and their reduced activities relative to the parental chromosome were caused by position effects. We note that our failure to detect effects on *40Fg* and *40Ff* in earlier study of 16 *lt*-variegating rearrangements (WAKIMOTO and HEARN 1990) suggested that these genes are relatively insensitive to position effects. Therefore, we believe that the severe effects of some of the Class IV derivatives were due to deletions of the *40Fg* and *40Fe* genes.

The complementation behavior of the derivatives with the distal genes, *40Fd, lt, 40Fc*, and *40Fa* allowed us to better distinguish the derivatives from one another. Three chromosomes, lt^{x13p20}, tx^{p5} and tx^{p15}, had severe effects on the *40Fd* gene. The remaining chromosomes partially or fully complemented a lethal *40Fd* mutation and therefore retained the gene. The strength of the effects of the derivatives on *40Fc*, the gene distal to *lt*, ranged from mild (72–88% viability), to intermediate (50–67%), to equivalent to a deletion of the locus (36–37%) (WAKIMOTO and HEARN 1990). Mutations of the *lt* and *40Fa* genes are both associated with lethal and cell autonomous visible phenotypes. Therefore, the effects of the derivatives on these genes could be assayed by measuring viability over a lethal allele and by scoring for PEV of visible mutant phenotypes.

As shown in Table 3, the derivative chromosomes differed in their ability to complement a lethal allele of the *lt* gene. To determine if the low levels of *lt* expression from the derivatives were due to severe position effects on the gene, we assayed pigmentation in the Malpighian tubules. This assay provided a more sensitive measure of lt^+ levels because individual polytene cells of the tubule can be monitored for *lt*-dependent pigmentation. The cell counts shown in Table 4 were consistent with the lethal complementation data in demonstrating a difference between the parental chromosome and all of the derivatives. Moreover, these data showed that the effects of some derivatives could be attributed to strong variegated position effects. Southern blot analysis showed that all but two of the derivatives retain sequences that hybridize to the *lt* gene (data not shown). The tx^{p5} and tx^{p15} strains that had the smallest number of pigmented cells did not retain the *lt* sequences. The two derivatives, tx^{p5} and tx^{p15}, were also associated with the most extreme effects on several of the other 2Lh genes and were likely to be the largest 2Lh deficiencies among the Class IV chromosomes.

Flies heterozygous for parent translocation, *T(2;3)* lt^{x13} $P[ry^+\ w^+]$, and a lethal allele of *40Fa*, had reduced viability (82%) relative to a control class and exhibited a cell autonomous roughened eye phenotype. Our previous studies suggested that the *40Fa* gene activity correlated with *lt* activity in *lt*-variegating rearrangements (WAKIMOTO and HEARN 1990). We therefore expected the *40Fa* gene to be similar in its behavior to the lt^+ gene and severely affected in all of the Class IV derivatives.

TABLE 3

Complementation behavior of *T(2;3)lt^{x13}*P[*ry*$^{+}$*w*$^{+}$] and Class IV derivatives

Translocation	2Lh Gene						
	40Fg	*40Fe*	*40Ff*	*40Fd*	*lt*[a]	*40Fc*	*40Fa*[b]
lt^{x13}	0.97 ± 0.06	1.12 ± 0.04	0.79 ± 0.04	1.22 ± 0.19	0.65 ± 0.13	1.22 ± 0.13	0.82 ± 0.07
tx^{p31}	0.66 ± 0.20	0.40 ± 0.04***	0.00 ± 0.00***	1.03 ± 0.13	0.08 ± 0.02***	0.75 ± 0.08**	1.10 ± 0.07
tx^{p18}	0.08 ± 0.05***	0.00 ± 0.00***	0.00 ± 0.00***	1.03 ± 0.10	0.19 ± 0.08**	0.67 ± 0.19**	0.94 ± 0.09
tx^{p1}	0.13 ± 0.04***	0.06 ± 0.02***	0.00 ± 0.00***	0.98 ± 0.04*	0.04 ± 0.01***	0.50 ± 0.18**	1.02 ± 0.06
tx^{p19}	0.40 ± 0.12**	0.14 ± 0.02***	0.00 ± 0.00***	0.92 ± 0.06*	0.05 ± 0.01***	0.88 ± 0.06*	1.05 ± 0.09
lt^{x13p89}	0.33 ± 0.06***	0.15 ± 0.04***	0.00 ± 0.00***	0.68 ± 0.07***	0.05 ± 0.02***	0.80 ± 0.10**	1.16 ± 0.02*
lt^{x13p20}	0.53 ± 0.10**	0.29 ± 0.11***	0.01 ± 0.01***	0.05 ± 0.02***	0.03 ± 0.01***	0.72 ± 0.10**	1.11 ± 0.07*
tx^{p5}	0.41[c]	0.04	0.00	0.08	0.00	0.37	0.79 ± 0.10
tx^{p15}	0.12 ± 0.06***	0.00 ± 0.00***	0.00 ± 0.00***	0.05 ± 0.02***	0.00 ± 0.00***	0.36 ± 0.06***	1.07 ± 0.10

The viability of individuals heterozygous for the translocation and a mutant allele of the 2Lh gene is expressed relative to the viability of individuals heterozygous for the translocation and balancer chromosomes. The ratios were compared with those obtained from crosses with the parental line. Values are means ± SE. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

[a] Survivors show a mutant light phenotype.

[b] Some survivors show a mutant roughened-eye phenotype.

[c] Only a single trial was performed for the *tx^{p5}* crosses due to low viability of the stock. More than 150 progeny were scored for each test.

TABLE 4

Comparison of the effects of Class IV derivatives on *lt*$^{+}$ and *w*$^{+}$ activity

Translocation	No. of pigmented *lt*$^{+}$ cells/individual[a]	Level of *w*$^{+}$ pigmentation[b]
lt^{x13}	10.8 ± 1.0	orange
tx^{p31}	5.9 ± 1.2	white
tx^{p18}	7.5 ± 1.5	yellow mottled
tx^{p1}	3.6 ± 0.6	yellow mottled
tx^{p19}	2.0 ± 0.8	yellow mottled
lt^{x13p89}	1.1 ± 0.3	orange
lt^{x13p20}	2.1 ± 0.7	yellow mottled
tx^{p5}	0.5 ± 0.3	white mottled
tx^{p15}	0.1 ± 0.1	white mottled

Values are means ± SE.

[a] Number of cells showing pigment granules from a total of 60 cells of the Malpighian tubule counted from a minimum of 15 larvae that were *T(2;3)*/lt^{1}*.

[b] Eye pigmentation was scored in *w^{1118}*; *T(2;3)*/+* flies.

Surprisingly, there was no correlation between the activity of *lt* and *40Fa*. All of Class IV derivatives showed only a weak effect on the *40Fa* gene. Individuals heterozygous for a lethal allele of *40Fa* and any one of the derivatives were fully viable, but in all cases, some individuals showed the variegated roughened eye phenotype characteristic of the parent chromosome. A possible explanation for this observation will be presented in the discussion.

In summary, the complementation behavior of the Class IV derivatives with the 2Lh mutations was consistent with the hypothesis that these chromosomes carry deletions with different breakpoints within the heterochromatin. The *lt* gene was sensitive to changes in the heterochromatin at the breakpoint. Conversely, the activity of the distal most 2Lh gene, *40Fa*, did not appear to be differentially affected by the derivatives. Thus, these 2Lh genes differ in their dependence on heterochromatin.

Cytological analysis of *T(2;3) lt^{x13} P*[*ry*$^{+}$ *w*$^{+}$] and Class IV derivatives: Examination of salivary gland polytene chromosomes and diploid neuroblast mitotic chromosomes showed that the overall structure of the parental translocation chromosome was maintained in all of the Class IV derivative lines. Thus, *P*-element mutagenesis did not induce detectable chromosome rearrangements other than the changes at the heterochromatin-euchromatin junction described below.

To determine if visible changes had occurred in the mitotic heterochromatin, chromosomes were examined after DAPI staining, N-banding and FISH with labeled probes that recognize specific subdomains of 2Lh. Four cytologically distinct regions designated, *h35-h38*, can be resolved in 2Lh using these techniques (DIMITRI 1991; PIMPINELLI *et al.* 1995) (Figure 2). All of the 2Lh genes relevant to this study are located within or just proximal to *h35* (DIMITRI 1991). The heterochromatic breakpoint of *T(2;3) lt^{x13} P*[*ry*$^{+}$ *w*$^{+}$] is within *h37*, displacing part of this DAPI positive band, and the more distal *h36* and *h35* bands to the distal euchromatin of Chromosome *3* (WAKIMOTO and HEARN 1990) (Figure 2, A and B). We estimate that the displaced 2Lh is ~5 mb in size, based on its similarity in size to Chromosome *4* (LOCKE and MCDERMID 1993) in mitotic chromosome preparations.

The Class IV derivative chromosomes were examined after DAPI staining, and by FISH analysis to determine if they retained the 2Lh sequences that hybridize to the 1.705 satellite sequences (Figure 2C) (BONACCORSI and LOHE 1991) and the *Doc* retrotransposon (PIMPINELLI *et al.* 1995). All eight derivatives showed the following properties. First, they deleted portions of 2Lh. Second,

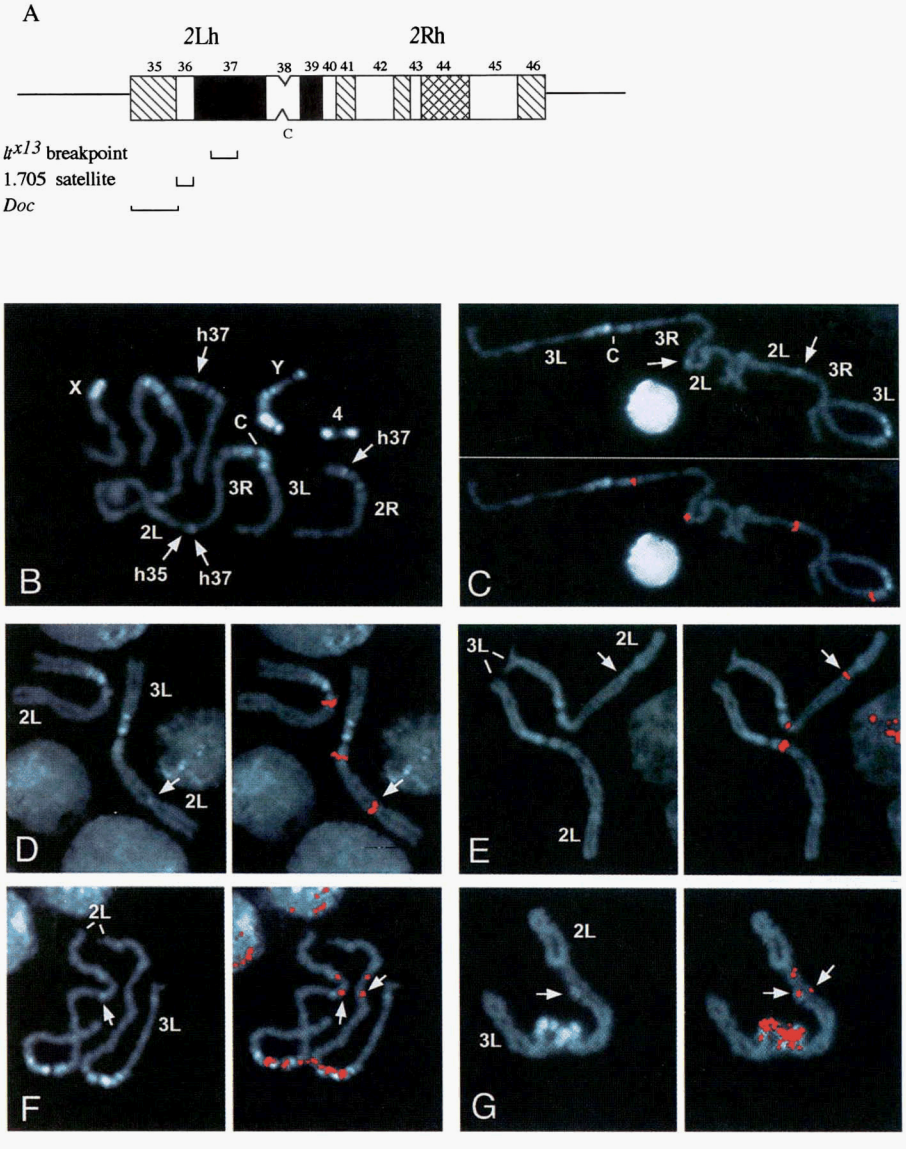

FIGURE 2.—Cytogenetic analysis of *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] and its derivatives. (A) Chromosome *2* heterochromatin contains the *h35-h46* bands as resolved by N banding and intensity of DAPI staining (DIMITRI 1991). The white regions are N bands that do not stain with DAPI. Hatched regions show weak staining with DAPI while the black regions are strongly DAPI positive. The position of the 2Lh breakpoint of *T(2;3) lt*^{x13} is shown by the bracket below the map. Other brackets mark the site of hybridization of the 1.705 satellite (LOHE *et al.* 1993) or the *Doc* element (PIMPINELLI *et al.* 1995) within *2*Lh. Sites within *2*Rh are not shown. (B) A DAPI stained prometaphase chromosomes from male homozygous for the *T(2;3)lt*^{x13} translocation. A portion of the the DAPI-positive *h37* block (arrows) is retained on both halves of the translocation. Labels indicate the centromere (c), each chromosome of the complement (*X, Y, 2, 3, 4*) with left (L) and right (R) arms designated. (C-E) The relevant half of the translocation of *T(2;3)lt*^{x13} heterozygotes is shown in two images so the DAPI bands can be aligned with hybridization signals (pseudocolored red). Arrows point to the *h35-h37* regions. The 1.705 satellite hybridization signals is visible on both chromosomes from individuals of the following genotypes: (C) *T(2;3)lt*^{x13}/*T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$], (D) *T(2;3)lt*^{x13}/*tx*^{p31}, and (E) *T(2;3)lt*^{x13}/*tx*^{p18}. A second signal is present on all chromosomes and corresponds to region *h57* in *3*R heterochromatin. (F) Multiple sites of hybridization to the *Doc* element, including a site in 2L euchromatin and one in *h35* (arrow). Note that a Doc signal of similar size is seen on both chromosomes in *T(2;3)lt*^{x13}/*tx*^{p19} heterozygotes. (G) *Doc* hybridization to *T(2;3)lt*^{x13}/*tx*^{p15} heterozygotes.

all of the derivatives were large deletions. Each removed the DAPI-positive *h37* region. Most if not all of *h36* band was also deleted, as shown by FISH analysis using the 1.705 satellite probe. Figure 2, C and D, shows these results for the *tx*^{p31} and *tx*^{p18} chromosomes. The derivatives maintained at least a portion of the *h35* band, as shown by the hybridization to the *Doc* element (Figure 2, E and F). Thus, as much as half the heterochromatin, including the large blocks of satellite sequences in the displaced *2*Lh were deleted as a result of the *P* transposase mutagenesis. These results suggest that the differences in the complementation behavior of the derivatives may be attributed to differences in the breakpoints within or near *h35*.

Molecular analysis of the Class IV derivatives: Molecular characterization of the Class IV derivatives was used to test the prediction that these chromosomes represented a series of overlapping deficiencies that shared a breakpoint near the *P* element. The structure of the *P*[*ry*$^{+}$ *w*$^{+}$] element and flanking regions was examined in each derivative by genomic Southern blot analyses. DNA from flies heterozygous for the *lt*^{l} mutation and *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] parental translocation or a derivative chromosome was probed with sequences that hybridized to the *P*[*ry*$^{+}$ *w*$^{+}$] element (Figure 3A). Only the parental *P* element was present in the derivatives, indicating that there was no transposition of the *P* element to new sites. As shown in Figure 3B, fragments that hybridize to *ry* probe sequences were unchanged in all the derivatives except *tx*^{p31} which suffered a deletion internal to the *P* element. The 6.8-kb *BamH*I fragment that flanks the 3′ *P* end in the parent was retained in all lines. Additional Southern blots with *Eco*RI and *Xba*I digested DNA provided further evidence that the 3′

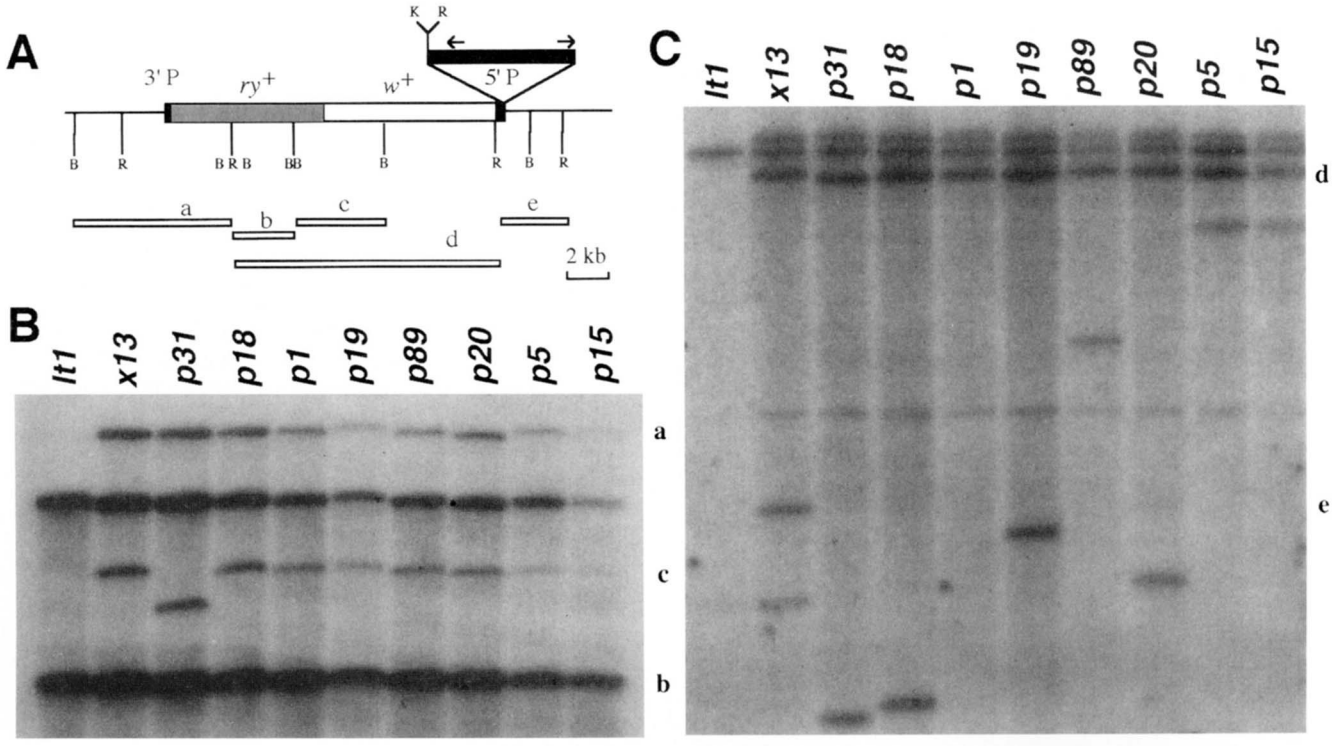

FIGURE 3.—Genomic Southern blot analysis of the structure of the *P* element and flanking regions in the *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] parental and Class IV derivative lines. (A) The *P* element in the parent chromosome contains the *ry*$^{+}$ gene (shaded box), *w*$^{+}$ gene (white box) and P element ends (black boxes). A 550-bp region containing the 5′ *P* end is expanded to show the position and orientation of the primers used to amplify the flanking genomic sequences. The *Bam*HI (B), *Eco*RI (R), *Kpn*I (K) and *Xba*I (X) recognition sites within the transposon and its flanking DNA are noted. Fragments, labeled a–e, correspond to the bands detected in the genomic Southern blots shown in B and C. For B, DNA extracted from *lt*^{l} homozygotes, or heterozygotes that carried the *lt*^{l} chromosome and the parental or derivative translocation was digested with *BamH*I then probed with *ry*$^{+}$ sequences. Only the *tx*^{p31} derivative shows a detectable difference due to a deletion internal to the transposon. For C, DNA was digested with *Eco*RI and probed for both *w*$^{+}$ and 5′ *P* sequences. All of the derivative lines show changes in the size of the fragment flanking the 5′ *P* element end.

flank was unchanged. In contrast, the parental *Eco*RI fragment that contains the 5′ end of the *P* element and its flanking genomic sequences was altered in all of the derivatives (Figure 3C). This observation, together with the genetic data, allowed us to infer the orientation of the *P* element shown in Figure 3A. The regions flanking the 5′ *P* end in each of the derivatives contained recognition sites for two or more common restriction enzymes within 15 kb of the *P* element terminus. This observation suggests that the genomic DNA at the breakpoint was composed of complex DNA, rather than long arrays of simple sequence DNA.

Retention of *P* sequences in the derivatives allowed us to compare the genomic sequences at the distal breakpoint of the parental and derivative translocations. Flanking sequences from the parental chromosome were amplified by inverse PCR (OCHMAN *et al.* 1989) using primers complementary to the 5′ *P* element end. These PCR products were used to probe Southern blots containing DNA isolated from the *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] parental strain and each of the derivative lines. The *P* element in the parental chromosome was inserted into the euchromatin at *97B*. When the 1.2-kb fragment of genomic DNA that flanked the 5′ *P* end in this chromosome was PCR amplified and used as a probe to *Eco*RI-*Xba*I digested DNA from *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$]/*lt*^{l} individuals, two strongly hybridizing bands of 6 and 3.5 kb were detected (Figure 4A). The larger band is derived from the *lt*^{l} chromosome. The smaller fragment, attributed to the parent chromosome was absent in all of the Class IV derivatives. This result provided strong molecular evidence that the derivative chromosomes carried deletions that begin within 1.2 kb of the 5′ *P* end.

We also amplified sequences flanking the 5′ *P* element end from four derivative chromosomes to determine if the sequences adjacent to the new breakpoints were single copy or repetitive in nature. The PCR products amplified from *tx*^{p31}, *tx*^{p1}, *lt*^{x13p89} and *lt*^{x13p20} recognized multiple bands in genomic DNA when used as probes for Southern blots. Figure 4, B–D, shows the Southern blots probed with the PCR products amplified from *tx*^{p1}, *lt*^{x13p89} and *lt*^{x13p20}, respectively. Each PCR product yielded a characteristic hybridization pattern, suggesting that the *P* element was adjacent to different repetitive elements in each of the lines. The data are consistent with the conclu-

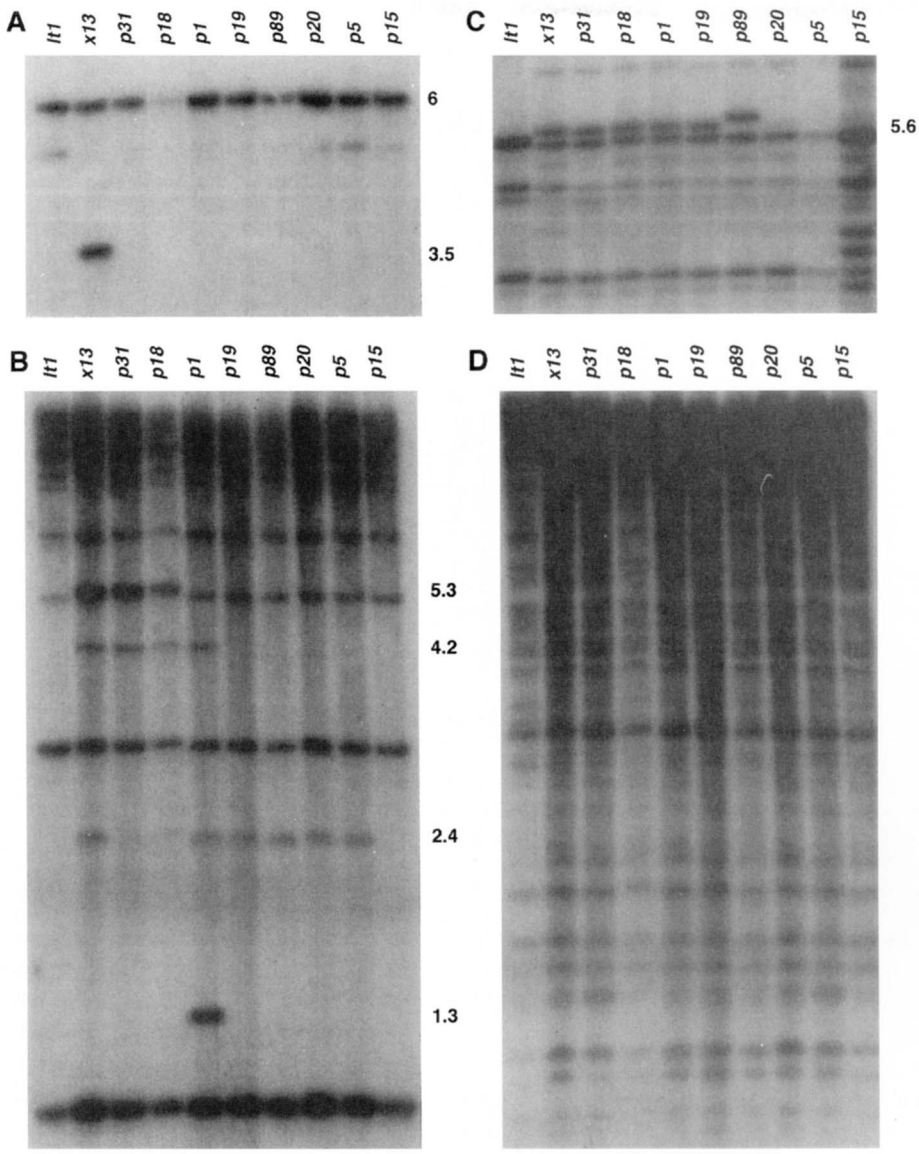

FIGURE 4.—Southern blot analysis of the nature of the genomic sequences flanking the 5′ *P* element end in the *T(2;3) lt*^{x13} *P*[*ry*$^{+}$ *w*$^{+}$] parent and derivatives. Genomic DNA was extracted from *lt*^{1} homozygotes or *lt*^{1} heterozygotes that also carried the parental or derivative translocation. DNA from females was used in all cases, except lanes 1–9 in C, which contain male DNA. In A, B, and D, DNA from the *tx*^{p18} strain was underloaded. Sizes of the relevant fragments are noted to the right of each panel. (A) *Eco*RI-*Xba*I DNA was probed with the labeled PCR product amplified from the parental translocation. A small band of ~3.6 kb is present only in the parental line. The 6-kb and the faint 5.2-kb band are characteristic of the *lt*^{1} chromosome. (B) *Kpn*I-digested DNA was probed with the PCR product amplified from the *tx*^{p} chromosome. The 5.3-kb band present only in the parental, *tx*^{p31} and *tx*^{p18} lines, comigrates with a band that remains constant, accounting for darker 5.3-kb signal in these lines. The comigrating fragments were resolved into two bands on other blots (data not shown). (C) *Eco*RI-*Xba*I-digested DNA probed with the PCR product from *lt*^{x13p89}. The 5.6-kb fragment is present in some but not all strains and this fragment is polymorphic in the *lt*^{x13p89} strain. The additional bands in the *tx*^{p15} lane are probably due to the *w*^{1118} marked *X* chromosome present only in this line. (D) *Kpn*I-digested DNA was probed with PCR product amplified from the *lt*^{x13p20}.

sion that these Class IV deletions terminated at different sites within 2Lh.

The PCR products amplified from the *tx*^{p1} and *lt*^{x13p89} derivatives were informative for assessing the relative sizes of the Class IV deletions. As shown in Figure 4B, when flanking sequences amplified from *tx*^{p1} were used to probe DNA from each of the lines on a Southern blot, multiple *Kpn*I fragments were recognized. Four fragments were present in some, but not all lines. A 1.3-kb band was present only on the *tx*^{p1} chromosome; this band also hybridized to probes to the 5′ *P* sequences (data not shown) and corresponded to the fragment that flanked the *P* element. The 5.3-, 4.2- and 2.4-kb bands were present in the parent chromosome but absent in some of the derivatives, suggesting that these fragments mapped to 2Lh. For example, the 4.2-kb band was present in the parent and three of the derivatives but absent in the remaining five derivatives. This pattern suggested that there was less 2Lh material deleted from the *tx*^{p31}, *tx*^{p18} and *tx*^{p1} chromosomes than the other chromosomes. The *tx*^{p31} and *tx*^{p18} derivatives were the only chromosomes that also retained the 5.3- and 2.4-kb bands, suggesting that these chromosomes contained the smallest deletions. Conversely, the *tx*^{p15} chromosome did not retain any of the bands, indicating that it was the largest deletion. A consistent conclusion was reached when the PCR product amplified from the *lt*^{x13p89} chromosome, was used as a probe for Southern blots (Figure 4C). In this case, a 5.8-kb band that coincides with the 5′ *P* flank of *lt*^{x13p89} was specific to this chromosome. This probe recognized a 5.6-kb band in the parental chromosome and some of the derivative lines. These bands were absent in the *lt*^{1}, *lt*^{x13p20}, *tx*^{p5} or the *tx*^{p15} chromosomes. The hybridization pattern suggested that the *lt*^{x13p89} deficiency was larger than *tx*^{p19} and smaller than the *lt*^{x13p20} deficiency.

The molecular data were consistent with the idea that the derivatives represented a series of successive deficiencies that could be ordered on the basis of relative size. The order from the smallest deficiency to the largest in size was:

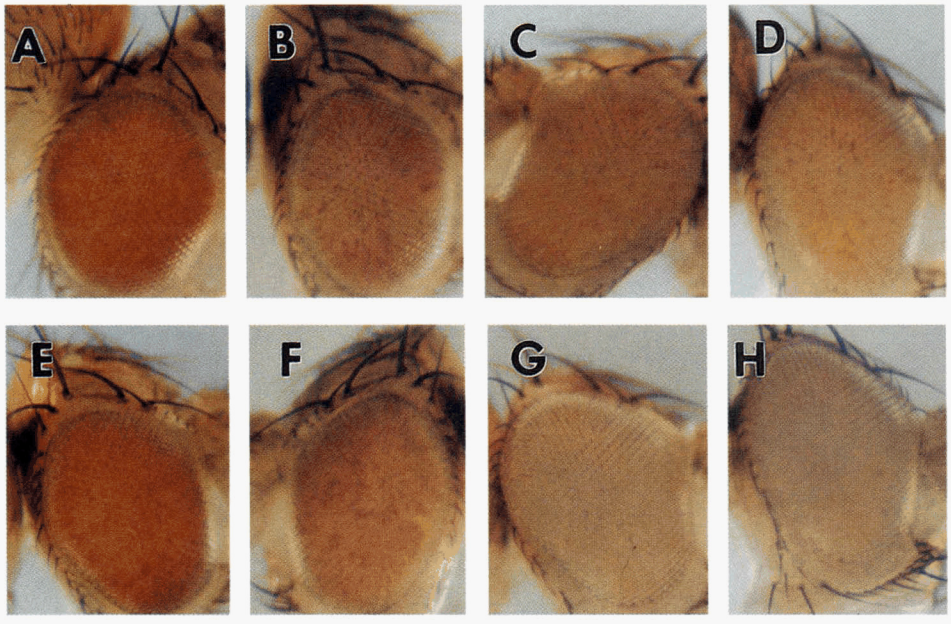

FIGURE 5.—Comparison of the eye color phenotypes conferred by the *white* gene in the transposon in the *T(2;3)* lt^{x13} $P[ry^+ w^+]$ parental and derivative lines. The photos show the eye color of male flies in a w^{1118} background. The homogeneous orange eye color of the parental *T(2;3)* lt^{x13} $P[ry^+ w^+]$ line is shown in A. Flies from each of the derivative are arranged with smallest to the largest deletions, from B–H as: (B) tx^{p18}, (C) tx^{p1}, (D) tx^{p19}, (E) lt^{x13p89}, (F) lt^{x13p20}, (G) tx^{p5} and (H) tx^{p15}.

$$\frac{tx^{p31}}{tx^{p18}} < tx^{p1} < tx^{p19} < lt^{x13p89} < lt^{x13p20} < tx^{p5} < tx^{p15}$$

These mapping data, in conjunction with the genetic complementation data, suggested that the amount of heterochromatin in *cis* to the *lt* gene correlated with its level of activity. That is, chromosomes that carried the smallest deletions of heterochromatin had weaker effects on the lt^+ gene (Table 4) when compared to those with the largest deletions of *2*Lh. This general trend is also observed for the *40Fd* gene (Table 3).

Position effect variegation of the euchromatic w^+ gene was induced in Class IV derivatives: The *white* gene in the $P[ry^+ w^+]$ transposon carried on the parental chromosome conferred a homogeneous orange eye color in a w^- background (Figure 5A). One of the derivative chromosomes, tx^{p31} was deleted for a portion of the *white* gene in the transposon (Figure 2C) and resulted in a w^- phenotype. The other seven derivative strains showed white-variegated phenotypes (Figure 5, B-H). Several lines of evidence suggested that the variegation of the *white* gene was due to position effects. As shown in Figure 3C, Southern blot analysis suggested that the *white* gene in the transposon was intact, because the *Bam*HI, *Eco*RI and *Xba*I restriction map was identical to that of the parent transposon. In addition, flies carrying any of these derivative chromosome and the $P[ry^+\Delta 2\text{–}3]$ (*99B*) source of *P* transposase showed somatic sectoring in the eye; we observed orange or dark red patches that were darker than the eye color in the derivative line. We also recovered dark-eyed progeny from flies carrying the derivative and $P[ry^+\Delta 2\text{–}3]$ (99B), indicating that variegating-inducing effect was also revertible in the germline.

Differences were observed among the derivative lines in the severity of *w*-variegation. Flies bearing lt^{x13p89} (Figure 5E) showed a weak variegated phenotype, with the overall level of pigmentation similar to that of the parental strain (Figure 5A). All other derivatives resulted in reduced and mottled eye pigmentation. While flies bearing four of the derivatives had similar, yellow-mottled phenotypes (Figure 5, B–D and F), those that carried the tx^{p5} and tx^{p15} (Figure 5, G and H) chromosomes had nearly white eyes with few pigmented facets. The extreme-variegated phenotypes of the tx^{p5} and tx^{p15} flies allowed us to conclude that the severity of the repressive effect on *w* did not correlate with the size of the block of heterochromatin inducing PEV. Moreover, as shown in Table 4, there was no strict inverse correlation between the amount of lt^+ and w^+ gene activity supported by any one region of heterochromatin.

DISCUSSION

We isolated a series of overlapping deletions that offered advantages for the study of heterochromatin and PEV. This set of overlapping deficiencies shared the same euchromatic breakpoint and differed only in the heterochromatin at the heterochromatin-euchromatin junction. Using these chromosomes, we were able to compare directly the effects of a particular block of heterochromatin on the expression of seven different heterochromatic genes and the euchromatic $white^+$ gene. Although the *2*Lh genes shared a requirement for heterochromatin, they responded differently to de-

letions of 2Lh. Additionally, the severity of the position effect on the euchromatic *white*$^+$ gene was not correlated with the amount of heterochromatin adjacent to this gene.

There are several unexpected properties of the heterochromatic deficiencies generated by *P* mutagenesis. In our screens, we selected only for decreased activity of the *lt*$^+$ or *tx*$^+$ genes so deletions that removed the entire *P* element could have been recovered. Surprisingly, all eight of the derivatives that were molecularly characterized, retained *P* element sequences and had a proximal breakpoint within 1.2 kb of the 5′ *P* end. Moreover, seven of the eight deficiencies retained an intact *P* element that carried the *w* gene. This gene provided an excellent genetic marker for examining the effects of heterochromatin on PEV at the euchromatic breakpoint. The *P* element also provided a molecular marker for mapping heterochromatic breakpoints. Finally, the cytological data suggested that the 2Lh breakpoints of the derivatives were within or just proximal to the *h35* band. This result suggests that there may be a bias inherent in the mechanism of *P* mutagenesis, such that breakpoints in *h37* and proximal *h36* were not recovered.

The large heterochromatic deletions were recovered at a low frequency of $\sim 10^{-4}$. The mechanism of deletion formation is unknown. Certainly the proximal breakpoint of the Class IV derivatives was generated by transposase activity at the 5′ *P* element end. Although we did not detect significant *P* element sequence homology other than the *P*[*ry*$^+$ *w*$^+$] element in our strains, the distal, heterochromatic breakpoint of the deficiencies may have also been generated by *P* transposase (McCarron *et al.* 1989; Duttaroy *et al.* 1990; Engles 1990). Alternatively, the distal breakpoints may have resulted from non-*P* transposase induced chromosome breakage, *e.g.*, as the result of sporadic breakage or by the mobilization of other transposable elements.

The complementation patterns of the chromosomes described in this paper indicate that the 2Lh genes do not respond in identical ways to changes in the heterochromatin in *cis*. Several factors must be considered for evaluating why the 2Lh genes show apparent differences in sensitivity in the complementation assays. First, our ability to detect changes in complementation behavior of the gene on the rearrangement depends on the nature of the tester allele. As noted earlier, we are limited in the choice of tester alleles to assay expression of the two most proximal 2Lh genes. The *40Fg* and *40Fe* tester alleles are semilethal and are themselves quite variable in expressivity (Wakimoto and Hearn 1990), making it difficult to interpret the significance of the differences observed for the derivatives chromosomes. Second, 2Lh genes may differ in the level of gene product required to produce a detectable mutant effect. That is, the fly may not be equally sensitive to a change in activity of each gene. Finally, differences in complementation behavior may reflect differences in the sensitivity of the 2Lh genes to PEV. Wakimoto and Hearn (1990) did not detect a position effect on the two most proximal genes, *40Fg* and *40Fe*, in a study of 16 *lt*-variegating chromosomes, even in the presence of mutations that strongly enhanced PEV of other 2Lh genes. These results suggested that these genes were relatively resistant to position effects. We interpret the complementation behavior of the *tx*^{p31} chromosome isolated in this study as evidence that the *40Fe* gene is subject to variegated position effects. A position effect on this gene may be revealed only in those cases when a breakpoint maps close enough to the gene to reduce the block of heterochromatin beyond a certain size (Eberl *et al.* 1993). In contrast to the *40Fe* gene, the *40Ff* and *lt* genes appear to be relatively sensitive to PEV, as judged by the fact that they are severely affected in all of the derivatives, including the six derivatives that were recovered on the basis of the mutant taxi phenotype.

The underlying molecular mechanism to account for why the 2Lh genes depend on heterochromatin and why they may differ in this dependence remains a mystery. The effect of modifiers of PEV suggest that this dependence reflects a requirement for the proteins enriched in heterochromatin (Hearn *et al.* 1990). Heterochromatic sequences may function to increase the concentration of these protein factors in the vicinity of the heterochromatic genes. A block of heterochromatin may serve this purpose by providing actual binding sites for specific proteins required for each 2Lh genes. Alternatively, heterochromatin may generally mediate the association or pairing with other heterochromatic regions to bring the 2Lh genes into a nuclear compartment that has an appropriately high concentration of these protein factors. Differential sensitivity of the 2Lh genes to variegated position effects may be due to differences in the level of heterochromatic proteins required by each gene. Those that appear to be resistant to PEV may simply require a lower concentration of heterochromatin binding proteins. Some genes may compete more effectively than others for a limiting amount of proteins or may be in closer proximity to the particular heterochromatic sequences that bind these proteins.

The enigmatic behavior of the *40Fa* gene suggested that it was unique in its response to heterochromatin. This gene variegated in all of the *lt*-variegating rearrangements studied by Wakimoto and Hearn (1990) and in these cases, the severity of position effects on *40Fa* correlated with the effects on the *lt* gene. The *T(2;3) lt*^{x13} *P*[*ry*$^+$ *w*$^+$] and all of its derivatives show variegated expression of *40Fa*. However, the level of *40Fa* activity in the derivatives did not correlate with the level of *lt* activity, showing that *40Fa* activity was unaffected by the removal of heterochromatic sequences *in cis*. The euchromatic *97C* breakpoint is shared among all the derivatives. Thus, the *97C* region may be compatible with high levels of *40Fa* activity, while other euchro-

matic regions may not be. Alternatively, because *40Fa* gene is the distal-most 2Lh gene, it may reside a border or transition zone between heterochromatin and euchromatin. A gene at this border may have two types of *cis* regulatory sequences permitting gene activity in either a heterochromatic or euchromatic environment. Large blocks of heterochromatin may result in the use of the "heterochromatic" *cis* regulatory sequences. The derivatives that remove most of the heterochromatin may allow the *40Fa* gene to function at relatively normal levels using the "euchromatic" regulatory sequences. Variegation of *40Fa* may result from disturbance of this border zone by chromosome rearrangements. This model suggest that the *cis*-acting regulatory sequences that allow a gene to function in heterochromatin differ from those that allow it to function in euchromatin and that they may coexist within a single gene.

Trans-acting modifiers of PEV, such as the *Y* chromosome (BAKER and REIN 1962) and some *Su(var)* mutations (GRIGLIATTI 1991; HEARN *et al.* 1991) have reciprocal effects on the activity of euchromatic and heterochromatic genes. Therefore, one simple model is that the *cis*-acting heterochromatic sequences also might have reciprocal effect on the activity of these two types of genes. The *white*$^+$ gene in the *P* element carried on the derivatives allowed us to monitor the effects of particular regions of heterochromatin on both heterochromatic and euchromatic gene activity. Comparisons of the white-variegating phenotypes associated with the *lt*-variegating derivatives indicated that there is not a strict inverse correlation between the levels of activity of these two genes. Chromosomes that support similar levels of one of the genes, resulted in different levels of activity of the other gene. In addition, while the heterochromatic genes tended to have higher levels of activity as a result of more heterochromatin in *cis*, this was not the case for the *w*$^+$ gene. The white-variegated phenotypes of the *tx*^{p5} and *tx*^{p15} chromosomes highlight the fact that smallest blocks of heterochromatin can result in the most extreme repression of the *white*$^+$ gene.

Our data lead us to conclude that the differences in white-variegated phenotypes do not correlate with the quantity of heterochromatin at the breakpoint. Instead, we propose that the differences caused by the derivatives reflect the influence of a local heterochromatic environment. Several hypotheses have been developed to explain the structural basis for PEV on euchromatic genes (reviewed by JOHN 1988; HENIKOFF 1994). Our data are compatible with aspects of several of these models. There are particular regions within the heterochromatin that did not effectively inactivate the *white*$^+$ gene. This was demonstrated by the fact that flies carrying the *lt*^{x13p89} chromosome had nearly the same level of *white*$^+$ activity as did the parental strain. It has been suggested that small islands of euchromatin exist within the heterochromatin (MIKLOS and COTSELL 1990). The *lt*^{x13p89} chromosome may represent a case where a euchromatic gene is expressed properly because it is adjacent to one of these islands. Alternatively, the *white*$^+$ gene in this chromosome may be adjacent to a sequence that acts as a terminator of heterochromatin formation (TARTOF *et al.* 1984). The sequences that induced strong repression of the *white*$^+$ gene in the larger deletions of heterochromatin were still present in the *lt*^{x13p89} chromosome. It is interesting to note that the sequence adjacent to the *white*$^+$ gene in the *lt*^{x13p89} chromosome was low in copy number. Therefore, the repetitive nature and copy number of the sequence immediately adjacent to the variegating gene may be influencing the level of its activity (REUTER *et al.* 1985; DORER and HENIKOFF 1994).

This study has allowed the systematic comparison of the effects of heterochromatic subdomains on the activity of one euchromatic and seven heterochromatic genes. The recovery of successive deletions with clonable breakpoints greatly facilitated the molecular analyses by permitting us to determine the relative positions of the heterochromatic breakpoints and the nature of the sequences adjacent to the breakpoint. Further molecular characterization, including the long range mapping of the heterochromatin using the chromosomes described in this report, should be informative for deciphering the relationship between the genetic and cytological properties of heterochromatin, repetitive sequences and organizational features of heterochromatic DNA.

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