Chromosome Rearrangements Induce Both Variegated and Reduced, Uniform Expression of Heterochromatic Genes in a Development-Specific Manner

Karen S. Weiler and Barbara T. Wakimoto

Department of Zoology, University of Washington, Seattle, Washington 98195

Manuscript received January 3, 1998
Accepted for publication March 30, 1998

ABSTRACT

In *Drosophila melanogaster*, chromosome rearrangements that juxtapose euchromatin and heterochromatin can result in position effect variegation (PEV), the variable expression of heterochromatic and euchromatic genes in the vicinity of the novel breakpoint. We examined PEV of the heterochromatic *light* (*lt*) and *concertina* (*cta*) genes in order to investigate potential tissue or developmental differences in chromosome structure that might be informative for comparing the mechanisms of PEV of heterochromatic and euchromatic genes. We employed tissue pigmentation and *in situ* hybridization to RNA to assess expression of *lt* in individual cells of multiple tissues during development. Variegation of *lt* was induced in the adult eye, larval salivary glands and larval Malpighian tubules for each of three different chromosome rearrangements. The relative severity of the effect in these tissues was not tissue-specific but rather was characteristic of each rearrangement. Surprisingly, larval imaginal discs did not exhibit variegated *lt* expression. Instead, a uniform reduction of the *lt* transcript was observed, which correlated in magnitude with the degree of variegation. The same results were obtained for *cta* expression. These two distinct effects of rearrangements on heterochromatic gene expression correlated with the developmental stage of the tissue. These results have implications for models of heterochromatin formation and the nuclear organization of chromosomes during development and differentiation.

THE parameters that govern normal gene expression extend beyond a gene to its chromosomal and nuclear contexts. The discovery by Muller (1930) of position effect variegation (PEV) in *Drosophila melanogaster* first illustrated that chromosome rearrangements can have a profound effect on the expression of genes distant from the physical breakpoint. Further examples of PEV, as well as the discovery of position effects exerted upon transgenes in numerous experimental systems, support the conclusion that gene expression is sensitive to the chromosomal environment. The study of position effects provides an avenue to increase our understanding of the role of chromosome structure in the regulation of gene expression.

The underlying causes and resulting phenotypes of position effects are quite varied. Position effects in multicellular eukaryotes typically fall into two broad categories. The examples most simple to explain are those in which the regulatory elements of a resident gene interfere, in either a negative or a positive fashion, with those of a translocated gene or a transgene, resulting in temporal and/or tissue-specific changes in its expression pattern (reviewed by Wilson *et al.* 1990). Less well defined are examples of altered gene expression due to a change in chromatin environment. For example, in mammalian systems transgenes frequently insert into what is referred to as repressive chromatin, because of the resulting decreased or variegated transgene expression (reviewed by Martin and Whitelaw 1996). Repressive chromatin is present in both the heterochromatin and the euchromatin of mammalian chromosomes, although the nature, distribution, and variety of regions of repressive chromatin are still unclear. In Drosophila, however, chromosomal regions that induce variegated expression of transgenes correspond to only a few localized domains, most notably the pericentric heterochromatin and telomeres (reviewed by Weiler and Wakimoto 1995).

The study of position effects induced by chromosome rearrangements in Drosophila led to the establishment of PEV as a model system for studying how gene expression is influenced by higher order chromatin structure (reviewed by Grigliatti 1991; Reuter and Spierer 1992; Weiler and Wakimoto 1995). While stable effects on gene expression are typically associated with rearrangements having two euchromatic breakpoints, variegated gene expression (PEV) has been the hallmark of rearrangements that juxtapose the continually condensed pericentric heterochromatin with euchromatin, which decondenses during interphase of the cell cycle (Lewis 1950). Interestingly, the latter rearrangements can induce the variegated expression of genes that are euchromatic as well as genes that normally reside in

Corresponding author: Barbara T. Wakimoto, Department of Zoology, University of Washington, Kincaid Hall, Box 351800, Seattle, WA 98195-1800. E-mail: wakimoto@u.washington.edu

the heterochromatin. Although first suggested by cytological observations, the idea that a change in chromatin structure underlies PEV is strengthened by more recent genetic and biochemical studies (reviewed by Weiler and Wakimoto 1995). PEV has been shown to be sensitive to histone dosage (Moore *et al.* 1983) and mutations and compounds that affect histone acetylation (Mottus *et al.* 1980; Reuter *et al.* 1982; Dorn *et al.* 1986; Fanti *et al.* 1994). In some cases, PEV correlates with altered nuclease sensitivity (Wallrath and Elgin 1995).

Analyses of heterochromatic genes have suggested that PEV is, in addition, a model system for the study of the role of nuclear organization in gene expression. The study of *light* (*lt*)-variegating rearrangements (Wakimoto and Hearn 1990) revealed that heterochromatic genes require a heterochromatic environment for their normal expression, and implicated chromosomal interactions in the formation of heterochromatic compartments. Wakimoto and Hearn (1990) proposed that the association between displaced heterochromatin and pericentric heterochromatin within a compartment allowed for the exchange of heterochromatic proteins and/or the induction of the appropriate chromatin state necessary for normal heterochromatic gene expression. Support for this model has come from genetic analyses of position effects on the heterochromatic *rolled* gene (Eberl *et al.* 1993) and cytological studies showing the predicted variable association of a heterochromatic insertion and the pericentric heterochromatin (Csink and Henikoff 1996; Dernburg *et al.* 1996). Thus, chromosome rearrangements might also indirectly influence higher order chromatin structure, and consequently gene expression, through changes in chromosome organization within the nucleus.

Much has been learned about chromatin-induced position effects upon gene expression through work on silencing in yeast (reviewed by Pillus and Grunstein 1995). However, studies with *D. melanogaster* provide the opportunity to address the influences of tissue specificity and development on position effects. Specifically, the timing of the onset of variegated gene expression, as well as its plasticity, provide a window into the dynamics of higher order chromatin structure during development. In addition, determining if variegation of a gene shows tissue-specific differences should be enlightening as to the etiology of PEV. For practical reasons, the vast majority of studies of PEV have concentrated on chromosome rearrangements that cause mosaicism of pigmentation, most typically in the eye. Consequently, relatively little is known about variegated expression of genes in diverse tissue types or when, during development, the rearrangements first have their effect. Early cell lineage studies using two *white* (*w*)-variegating alleles, $w^{m258\text{-}18}$ and $w^{m264\text{-}58}$, suggested that in rare cases the eventual expression state of the *w* gene in ommatidial cells may be predetermined very early in development and clonally inherited (Becker 1961; Baker 1963; Janning 1970). This conclusion was by necessity indirect, as *w* gene expression cannot be monitored until late in eye development. Potentially more informative are analyses utilizing variegating alleles of genes that are ubiquitously expressed. Examination of whole larvae and adult flies bearing a variegating allele of the widely-expressed *purple* gene suggested that variegation may not begin until pupal development (Tobler *et al.* 1979; but see also Kim *et al.* 1996). A second study analyzing variegating alleles of the *Pgd* gene revealed a decrease in 6-PGD protein level in only a subset of larval tissues, suggesting tissue specificity (Slobodyanyuk and Serov 1987). However, in neither study was expression monitored at the cellular level. This caveat was recently addressed by the developmental studies of Lu *et al.* (1996) that employed a *hsp70-lacZ* reporter transgene, which was inducible in a wide variety of tissues and readily assayable for expression in individual cells. Two chromosome rearrangements were isolated which placed the *hsp70-lacZ* reporter near heterochromatin, and both caused variegation of heat shock-induced *lacZ* expression in embryonic, larval, and adult tissues. Interestingly, repression was more extensive in the larval eye-antennal imaginal disc than would have been predicted by the subsequent adult eye expression patterns, suggesting that a relaxation of repression (reactivation) occurred during eye differentiation.

Studies of *lt* variegation provide several advantages for investigating the dynamics of heterochromatin formation during development and in different tissues. First, the activation of *lt* transcription by heterochromatin reflects a normal function of heterochromatin, as opposed to its ability to repress euchromatic genes. Second, variegated expression of *lt* appears to reflect chromosome organization, thus yielding insight into nuclear architecture. Third, the *lt* gene is widely expressed throughout development (Devlin *et al.* 1990), allowing us to compare the effects of chromosome rearrangements on gene expression for multiple tissues and developmental stages.

This article describes an analysis of the effects of three chromosome rearrangements on *lt* expression in multiple tissues. We show that the relative strengths of the effects of the rearrangements on *lt* expression are consistent for a given *lt*-variegating allele in all tissues examined. Remarkably, we find that a rearrangement can either induce variegated expression or cause reduced nonvariegated expression. We have confirmed this result for a second heterochromatic gene, *concertina* (*cta*), suggesting that this may be a general property of PEV of heterochromatic genes. We attribute the differing effects of the chromosome rearrangements to the developmental stage of the tissue, and suggest that variegation of heterochromatic genes is restricted to differentiated cell types.

MATERIALS AND METHODS

Drosophila stocks and culture conditions: Stocks were maintained at 25° on standard cornmeal-molasses-agar medium. All of the mutations described in this study are listed within Flybase (http://flybase.indiana.bio.edu/).

Larvae for the Malpighian tubule assays and RNA *in situ* analyses were cultured at 25° under identical conditions of low crowding for each experiment. In order to eliminate potential sex differences only female larvae were used for the assays. For the *lt* and *cta* expression studies, larvae were derived from $Df(2L)lt^{X10}$ *Bc*/*SM1*, lt^{16} females, to effectively eliminate any maternal contribution to Malpighian tubule pigmentation. The paternal parent was *Canton S*, for the positive control cross, or carried the *TSTL14*, *Tb* balancer and either a *lt*-variegating allele or the *lt* deficiency, $Df(2L)lt^{X120}$. The *lt*/$Df(2L)lt^{X10}$ larval progeny were identified as *Bc* and Tb^{+} individuals.

Assays of Malpighian tubule pigmentation: Wandering third instar female larvae of the appropriate genotype were collected as described above. The Malpighian tubules were dissected in 0.7% NaCl, and stained in 0.1 μg/ml DAPI, 0.7% NaCl on a multiwell slide. For each larva, thirty contiguous cells from each posterior arm of the tubules were scored for the presence of pigment granules using UV illumination and ×100 magnification. Data for each genotype were accumulated from two to three experiments. Statistical analysis was performed using Statview 4.5 (Abacus Concepts, Inc., Berkeley, CA).

***In situ* hybridization to whole mount third instar larval tissues:** For a typical experiment, at least 15 wandering third instar female larvae of each genotype were processed. The anterior halves of the larvae were isolated and inverted in cold phosphate-buffered saline + 0.1% Tween-20 (PBT), in <30 min per sample. Each sample was immediately fixed in fresh 4% formaldehyde (EM grade; Electron Microscopy Sciences, Fort Washington, PA), 0.1% deoxycholate in PBT, for 20 min at room temperature, and then washed three times for 5 min each in PBT. The remaining pretreatments and hybridization procedure were a modification of the protocol of Tautz and Pfeifle (1989). The tissue samples were digested with 10 μg/ml proteinase K for 9 min, rinsed twice for 2 min each with 2 mg/ml glycine in PBT, rinsed twice for 1 min each in PBT, refixed for 20 min with 4% formaldehyde in PBT, washed 5 min in 2 mg/ml glycine in PBT, and finally washed twice for 5 min each in PBT. The tissues were then equilibrated in a 1:1 dilution of PBT and hybridization solution (50% formamide, 5× SSC, 50 μg/ml heparin, 0.1% Tween-20) for 5 min followed by 5 min in hybridization solution. Prehybridization was performed for at least 1 hr at 55° in hybridization solution supplemented with 100 μg/ml denatured salmon sperm DNA. Digoxigenin-labeled RNA probes were synthesized using digoxigenin-UTP (Boehringer Mannheim, Indianapolis), T3 or T7 polymerase, and 1 μg template [cDNA clones of the *light* (Devlin *et al.* 1990), *concertina* (Parks and Wieschaus 1991) and *string* (Edgar and O'Farrell 1989) genes] under standard reaction conditions, and reduced in length by limited alkaline hydrolysis. The tissue samples were hybridized in 500 μl hybridization solution supplemented with 100 μg/ml denatured salmon sperm DNA and denatured RNA probe (1/15 of the transcription reaction) overnight at 55°. Washes consisted of five 30-min incubations in hybridization solution at 55°, 20 min each in 80, 60, 40, and 20% hybridization solution in PBT at room temperature, followed by five 5-min washes in PBT.

Color detection of hybridized probe was performed by incubating the samples with alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim), which had been preadsorbed against unhybridized tissues, at 1:2000 dilution in PBT overnight at 4°. Following four 20-min washes in PBT, the tissues were rinsed twice for 5 min in freshly prepared staining buffer (100 mm NaCl, 50 mm $MgCl_2$, 100 mm Tris pH 9.5, 0.1% Tween-20, 0.1% levamisole) and then incubated in staining buffer supplemented with 4.5 μl/ml NBT and 3.5 μl/ml BCIP (Boehringer Mannheim) until the desired level of staining was achieved. The tissues were then stained for 10 min in 0.1 μg/ml DAPI, and mounted in 50% glycerol. The patterns of staining were visualized using a Nikon (Garden City, NY) Microphot microscope equipped with DIC optics.

Fluorescence detection of hybridized probe was performed as above except that the anti-digoxigenin antibody (at 1:500; Boehringer Mannheim) was unconjugated, the samples were washed in PBT four times for 30 min, incubated 4 hr in fluorescein-conjugated anti-sheep antibody (1:100; Jackson ImmunoResearch Labs., Inc., West Grove, PA) at room temperature, and washed again in PBT four times for 30 min. The tissues were mounted in 80% glycerol and examined using a ×60 (1.4 NA) objective on a Bio-Rad (Richmond, CA) MRC-600 confocal imaging system.

Assays of transcription in salivary glands: Salivary gland nuclei were visualized using DAPI staining and UV illumination. Each nucleus in the distal three-fourths of each gland was scored for the presence or absence of a focus of probe hybridization, using DIC optics. An average of 74 nuclei were scored per gland. The data for each lt^{var}/*Df* genotype derive from two experiments, and for lt^{+}/*Df* from six, with 10 to 20 glands assayed per experiment. Statistical analysis was performed using Statview 4.5 (Abacus Concepts).

RESULTS

The effects of *light* gene variegation on tissue pigmentation: The *lt* gene is essential for viability and required for the normal pigmentation of several tissues including the adult eyes and larval Malpighian tubules. More than 50 chromosome rearrangements that cause variegated expression of the *lt* gene in ommatidia have been isolated (Hessler 1958; Wakimoto and Hearn 1990). Three of these, lt^{G10}, lt^{X2}, and lt^{X13}, were selected for analyses of *lt* expression in additional tissues because they represented examples of severe, moderate, and weak *lt* variegation, respectively. The effects of these rearrangements on viability and eye pigmentation are included in Table 1, and the eye phenotype of flies heterozygous for an *lt*-variegating allele and lt^{1} is illustrated in Figure 1. The lt^{1} allele bears a hypomorphic mutation that results in a uniform, low level of pigmentation but does not reduce viability. We reasoned that a comparison of the extent of *lt* variegation in multiple tissues derived from individuals carrying each of these variegating alleles might allow us to detect potential tissue-specific differences in variegation behavior among the rearrangements. Additionally, commonalities in the variegation behavior of the three *lt*-variegating alleles, such as a tissue or developmental specificity, would likely be representative of *lt* variegation-inducing chromosome rearrangements, in general.

To compare the effects of the three *lt*-variegating rearrangements (collectively denoted lt^{var}) on viability

TABLE 1

Alleles of the *lt* gene used in this study

Allele	Genetic description[a]	Viability[b]	Eye phenotype[c]
lt^{G10}	In(2LR)40;59F3	Semiviable (3%)	Extreme variegation: uniform orange or a few red facets
lt^{X2}	In(2L)25D5;40+In(2LR)40;53A1,2	Semiviable (33%)	Moderate variegation: varies from a few red facets to large patches of red
lt^{X13}	T(2;3)40;97C	Viable (100%)	Weak variegation: red with scattered dark facets
lt^{X10}	Df(2L)lt^{-} cta^{-}	Inviable	Uniform orange
lt^{X120}	Df(2L)lt^{-} cta^{-}	Inviable	Uniform orange
lt^{1}	Uncharacterized spontaneous hypomorphic mutation	Viable	Uniform orange

[a] Data taken from Wakimoto and Hearn (1990).
[b] Indicates survival to adulthood, when the allele is heterozygous with a *lt* mRNA, most larvae survive to pupation.
[c] Observed when the allele was heterozygous with the lt^{1} allele.

and eye pigmentation to their effects on *lt* expression in the larval Malpighian tubules, we utilized the presence of pigment granules in individual tubule cells as an indicator of gene activity. The Malpighian tubules of female third instar larvae bearing a deficiency of the *lt* gene and an *lt*-variegating rearrangement were dissected and assayed as described in materials and methods. In $lt^{+}/Df(2L)lt^{X10}$ (abbreviated lt^{+}/Df) larvae bearing the lt^{+} gene situated on an unrearranged chromosome, 100% of the cells were pigmented. Individuals bearing two *lt* deficiency chromosomes, $Df(2L)lt^{X120}/Df$ survive until pupariation, making it possible to assay pigmentation in larvae deleted for the *lt* gene. The Malpighian tubules of these larvae were completely unpigmented. In contrast, the Malpighian tubules of larvae having a single *lt*-variegating allele were a mosaic of pigmented and unpigmented cells. A tabulation of the frequencies of pigmented cells in lt^{G10}/Df, lt^{X2}/Df and lt^{X13}/Df larvae is shown in Figure 2. The frequency of pigmented cells varied between individuals of the same genotype, as is characteristic of PEV. Malpighian tubules from lt^{G10}/Df larvae had very few or no pigmented cells per larva, indicating very severe variegation. The lt^{X2}/Df Malpighian tubules showed much greater pigmentation and a broad range in pigmentation frequency. The lt^{X13}/Df Malpighian tubules exhibited the weakest variegation, and showed less variability between larvae than the lt^{X2}/Df tubules. These results paralleled the effects of each rearrangement on viability (Table 1) and eye pigmentation (Figure 1): lt^{G10} greatly reduces viability and eye pigmentation; lt^{X2} causes a moderate reduction in viability and moderate eye variegation that is quite variable between individuals; and lt^{X13} does not reduce viability and has a weak effect on eye pigmentation.

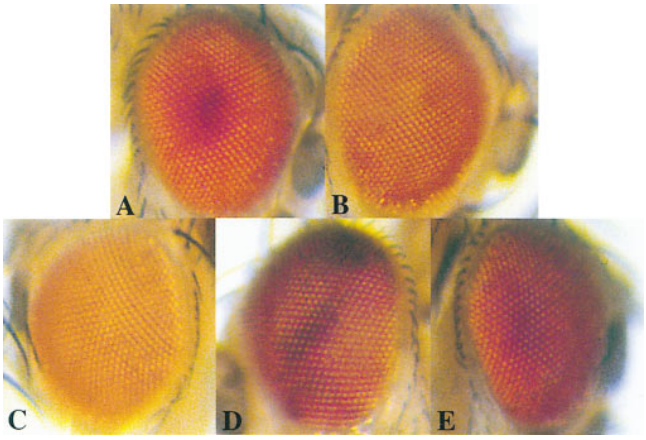

Figure 1.—Variegation of the *lt* gene in the adult eye. Chromosomes bearing an *lt*-variegating allele, lt^{+} or *Df(lt)* were assayed for *lt* gene activity in the adult eye when heterozygous with the *lt* hypomorphic allele, lt^{1}. (A) lt^{+}/lt^{1}; (B) Df/lt^{1}; (C) lt^{G10}/lt^{1}; (D) lt^{X2}/lt^{1}; and (E) lt^{X13}/lt^{1}.

Expression of the *light* gene in wild-type third instar larval tissues, as detected by RNA *in situ* hybridization: In order to assess the expression of the *lt*-variegating alleles in additional, unpigmented tissues, RNA *in situ* hybridization assays were undertaken. We first determined the extent and pattern of *lt* expression in tissues of wild-type third instar larvae. Single-stranded sense and anti-sense RNA probes were synthesized from the *lt* cDNA clone and hybridized to whole mount tissues. In agreement with the results of previous Northern analyses (Devlin *et al.* 1990), expression of the *lt* gene was detected using the anti-sense probe in all tissues examined (*e.g.*, larval brain, digestive system, circulatory system, imaginal discs, Malpighian tubules, fat body, gonads). In imaginal discs, cytoplasmic RNA appeared to be present in all cells, at similar levels (Figure 3A). The uniformity of staining was apparent during microscopic examination as the focal plane was adjusted through the full depth of the tissue (as well as by confocal microscopy, see below). However, since out-of-focus staining contributed to the intensity as observed in photographs, the staining appeared darker in areas of greater tissue thickness and where the tissue had folded over. Under the experimental conditions used in this study, the degree of staining appeared quantitative. Comparisons of staining levels were made only for tissues treated identi-

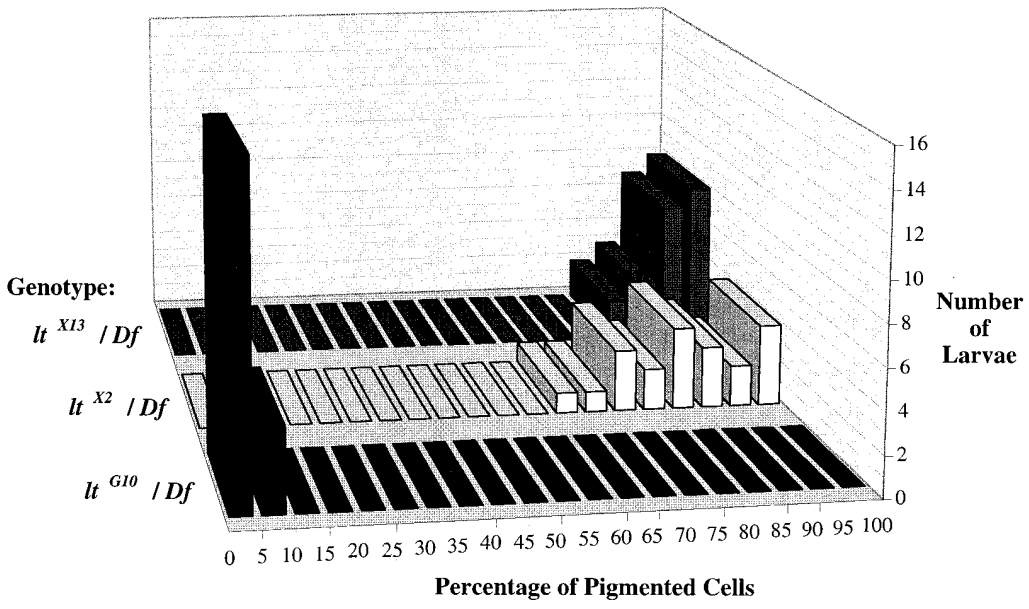

Figure 2.—Pigmentation of Malpighian tubule cells in larvae bearing *lt*-variegating chromosome rearrangements. The percentage of pigmented cells in tubules of individual larvae was assayed as described in materials and methods (60 cells per larva), and plotted as a histogram. Twenty third instar larvae were scored per genotype, from two or three experiments. Malpighian tubules from lt^+/Df larvae were fully pigmented, and those from $Df(2L)lt^{X120}/Df$ larvae were unpigmented. Data obtained for lt^{G10}/Df, lt^{X2}/Df, and lt^{X13}/Df differ significantly from lt^+/Df, using a Mann-Whitney *U*-test of median values ($P < 0.0001$). The lt^{X2}/Df and lt^{X13}/Df data are also significantly different ($P < 0.0068$).

cally within a single experiment. The sensitivity of this technique to detect different levels of *lt* transcript is illustrated by comparing the staining of tissues having two copies of the lt^+ gene (lt^+/lt^+ in Figure 3A) to those having a single copy (lt^+/Df in Figure 3B). Furthermore, as described below, certain *lt* genotypes yielding staining levels lower than one wild-type copy were easily distinguishable. In polytene tissues such as the gut, fat body, and salivary glands, a focus of nuclear RNA representing the site of transcription (O'Farrell *et al.* 1989; Boyd

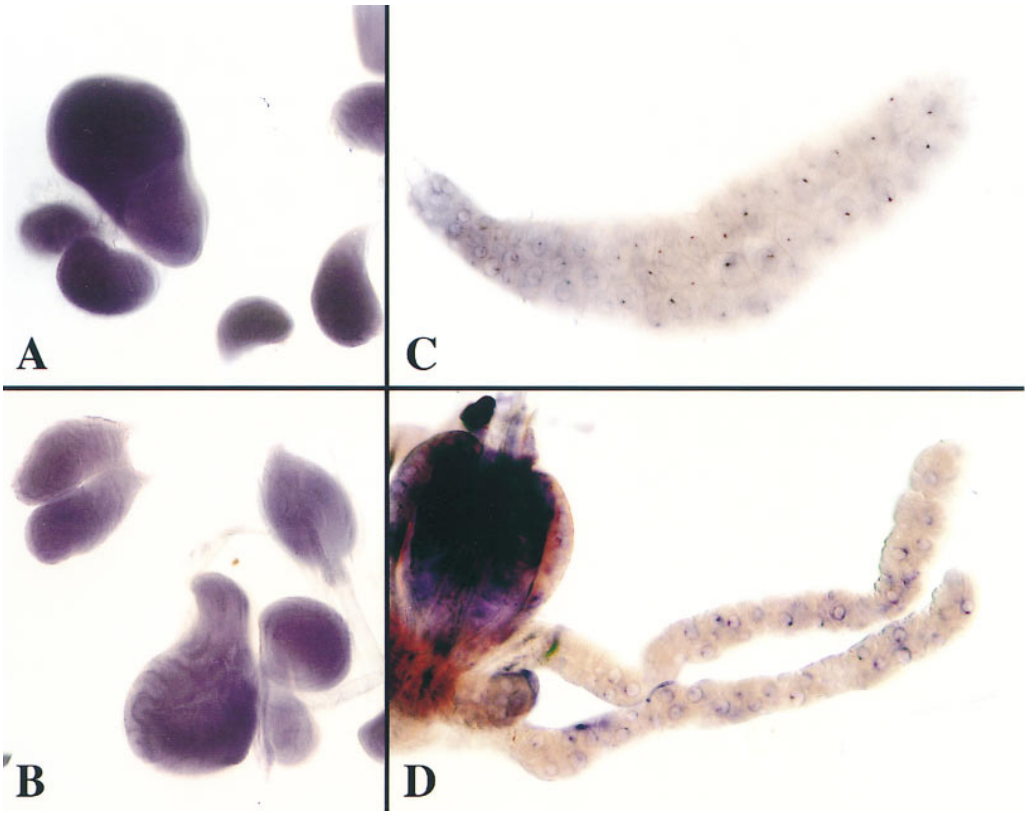

Figure 3.—Expression of the *lt* gene in tissues of late third instar larvae, as detected by *in situ* hybridization to *lt* RNA. Imaginal discs from lt^+/lt^+ larvae (A) exhibited a more intense cytoplasmic staining pattern than did those from lt^+/Df larvae (B). Nuclear staining was observed in polytene tissues such as the salivary gland from a lt^+/Df larva (C) and the foregut and gastric caecae from a lt^+/lt^+ larva (D). Many nuclei of the gut and gastric caecae are not in the plane of focus and therefore do not show a nuclear dot.

et al. 1991) was detected (Figure 3, C and D). The intensity of nascent transcript staining in salivary gland nuclei exhibited slight cell-to-cell variability, possibly reflecting differences in cell physiology and polyteny. Staining was rarely observed in the cytoplasm of the salivary gland cells. These results were in contrast to those obtained using an *lt* sense strand probe as a control, which showed no hybridization in the imaginal discs or the salivary glands (data not shown). As an additional control, larvae deleted for both copies of the *lt* gene were included in each experiment to reveal any nonspecific staining of imaginal discs. Nuclear foci were never observed for $Df(2L)lt^{X120}/Df$ salivary glands.

Salivary glands exhibit a mosaic *light* expression pattern, as a result of lt^{var} rearrangements: Having established the wild-type expression pattern of the *lt* gene, we then determined the effects of the lt^{var} rearrangements on *lt* expression in multiple unpigmented cell types. For these lt^{var} assays, the genotype of the larvae assayed was lt^{var}/Df so that only expression from the gene on the rearranged chromosome was monitored. A single focus of *lt* probe hybridization was observed within all nuclei of most lt^{+}/Df salivary glands (see Figures 3C and 4A; data in Figure 5). In contrast, the glands derived from larvae bearing a *lt*-variegating allele were a mosaic of cells having the nuclear staining and cells devoid of the nuclear staining (*e.g.*, lt^{G10}/Df in Figure 4B). We assessed the severity of variegation in this tissue for each *lt*-variegating allele by quantitating the fraction of cells expressing the *lt* gene. The percentage of expressing nuclei in individual salivary glands from lt^{+}/Df, lt^{G10}/Df, lt^{X2}/Df and lt^{X13}/Df larvae is illustrated in Figure 5. The effect of each rearrangement on *lt* expression in the salivary glands correlated in magnitude with its effect on *lt* expression in other tissues (compare with Figures 1 and 2). Only a few salivary gland cells from lt^{G10}/Df larvae expressed the *lt* gene. Salivary glands from lt^{X2}/Df and lt^{X13}/Df larvae showed a much higher frequency of staining nuclei than lt^{G10}/Df larvae, but were not statistically different from each other. As in the eye and Malpighian tubules, there was a large degree of variability between lt^{X2}/Df individuals in the frequency of salivary gland cells showing *lt* expression.

Imaginal discs exhibit reduced, nonvariegated expression of the *light* gene, as a result of lt^{var} rearrangements: As stated above, *in situ* hybridization to *lt* mRNA yielded a uniform staining pattern for lt^{+}/Df imaginal discs. When imaginal discs from larvae bearing any one of the three lt^{var} rearrangements were assayed, the staining pattern was less intense but still uniform. Tissues derived from at least four experiments were examined for each lt^{var}/Df genotype, and for three experiments all three lt^{var}/Df genotypes (and controls) were processed simultaneously and identically. Typical results from a single experiment in which individuals of all three lt^{var}/Df genotypes were processed identically are illustrated in Figure 6 for leg imaginal discs and Figure 7 for eye-antennal imaginal discs. The staining obtained for lt^{X13}/Df imaginal discs (Figures 6B and 7B) was strong but generally less intense than lt^{+}/Df imaginal discs (Figures 6A and 7A). The level of imaginal disc staining of lt^{X2}/Df larvae (Figures 6C and 7C) was intermediate between that of lt^{+}/Df larvae and $Df(2L)lt^{X120}/Df$ larvae (Figures 6E and 7E). The imaginal discs of lt^{G10}/Df larvae (Figures 6D and 7D) showed an extremely low level of staining, which was generally equivalent to or only slightly darker than that of $Df(2L)lt^{X120}/Df$ imaginal discs. Therefore, the chromosome rearrangements affected *lt* expression by reducing it to a level characteristic of each allele, but did not induce mosaicism of expression in these imaginal tissues. Uniform staining was observed upon examination of tissues at up to 400-fold magnification. We confirmed that cell-to-cell differences in gene expression could be detected using our *in situ* hybridization protocol by assaying imaginal discs for the *string* transcript, which is present in single cells scattered throughout the discs because of differences in stage of the cell cycle (data not shown;

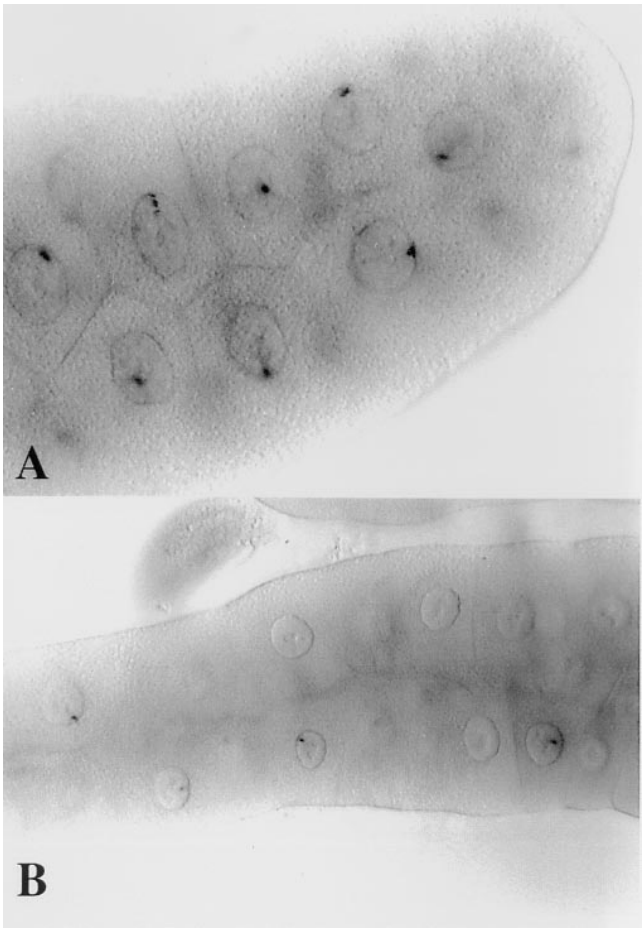

Figure 4.—Variegated *lt* expression in lt^{G10}/Df salivary glands. *In situ* hybridization to the polytene salivary glands revealed the nascent *lt* transcript in each nucleus of lt^{+}/Df glands (A). Only a subset of the cells appeared to be actively transcribing the *lt* gene within lt^{G10}/Df glands (B).

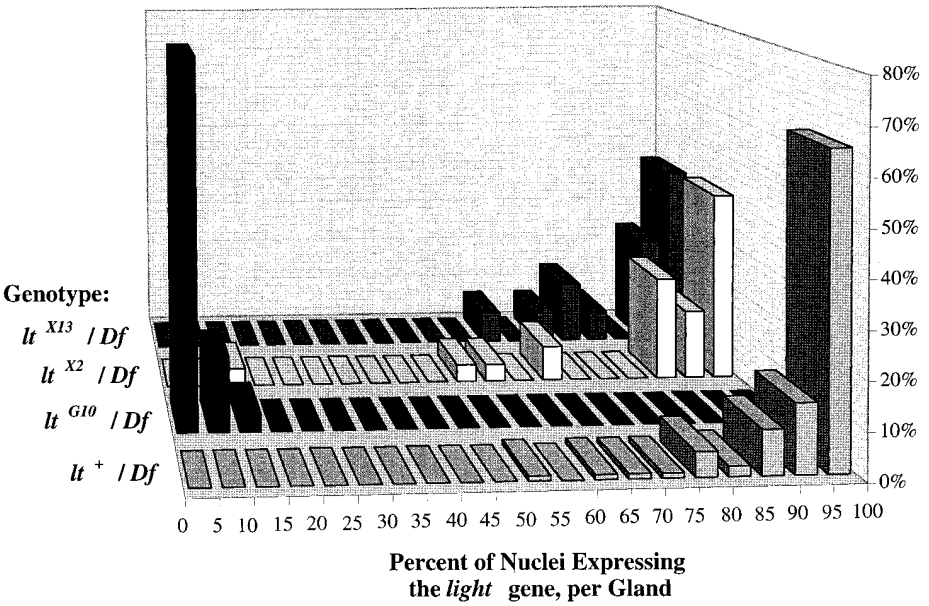

Figure 5.—Transcription of the *lt* gene in salivary glands. Expression of *lt* was assayed in salivary glands derived from the indicated genotypes, as described in materials and methods. The percentage of positive nuclei per gland is plotted as a histogram versus the percentage of total glands assayed, for each genotype. The data derive from 96 lt^{+}/Df glands, 25 lt^{G10}/Df glands, 26 lt^{X2}/Df glands, and 29 lt^{X13}/Df glands. The frequencies with which the *lt* nascent transcript was detected for lt^{+}/Df salivary glands are significantly different from those of lt^{G10}/Df glands ($P < 0.0001$), lt^{X2}/Df glands ($P < 0.003$), and lt^{X13}/Df glands ($P < 0.0113$), using a Mann-Whitney *U*-test of median values.

Milán *et al.* 1996). In addition, the accessibility of the imaginal discs to *lt* probe and antibody in these studies was supported by the observation that hemocytes associated with the interior pockets formed by folds of the eye-antennal disc epithelia showed a variegated *lt* RNA staining pattern (Figure 7F). It should be noted that within an experiment small differences in the intensity of staining among lt^{+}/Df imaginal discs were observed. A similar variability in staining between discs was observed for lt^{G10}/Df and lt^{X13}/Df larvae, whereas lt^{X2}/Df imaginal discs exhibited a broader range of staining. The greater variability among lt^{X2}/Df imaginal discs is likely a manifestation of the position effect.

It remained a formal possibility that the imaginal discs were composed of *lt*-expressing and *lt*-nonexpressing cells, but that the distinctions between cells were masked by convolutions of the tissue and/or associated hemocytes (which include the melanotic crystal cells; see Figure 7). Therefore, we performed *in situ* hybridization to *lt* mRNA in imaginal discs using fluorescence detection and confocal microscopy. We focused our analysis upon the portion of the eye-antennal disc that gives rise to the ommatidia, since *lt* variegation occurs in the adult eye. We examined 0.1-μm optical sections spanning the full thickness of the tissue for nine lt^{X2}/Df eye-antennal discs and three lt^{X13}/Df eye-antennal discs, but variegation was never observed. Rather, the staining was cytoplasmic and uniform across the disc, and was not observed within the nuclei. However, a comparison of the intensity of staining of lt^{X2}/Df and $Df(2L)lt^{X120}/Df$ tissues processed simultaneously showed that lt^{X2}/Df eye-antennal discs were more brightly stained (data not shown). As the lt^{X2}/lt^{1} adult eye phenotype frequently shows large patches of dark pigmentation (Figure 1D), a comparable pattern of variegation at the RNA level should have been readily apparent but was not detected. A representative section from an lt^{X2}/Df eye-antennal disc is shown in Figure 8. These results confirmed the results obtained using colorimetric detection of *lt* mRNA, in showing that *lt* RNA levels were uniform in the eye-antennal imaginal discs of *lt*-variegating strains.

The absence of mosaic expression in imaginal discs is observed for another variegating heterochromatic gene: To determine if the reduced nonvariegated mRNA staining pattern was unique to variegating alleles of the *lt* gene or was characteristic of other variegating heterochromatic genes, we assayed expression of the *cta* gene. The *cta* gene encodes a subunit of a G protein complex, and was identified as a maternal effect gene because it is required in female Drosophila for the normal development of their embryos (Parks and Wieschaus 1991). Using *in situ* hybridization to *cta* RNA, we determined that the wild-type *cta* gene was widely expressed in third instar larval tissues and showed uniform expression in the imaginal discs. The nascent *cta* transcript was also detected as a single focus of staining in salivary gland nuclei. Variegation of the *cta* gene in ovaries was initially detected as a variable reduction in progeny yield from individual females carrying a single wild-type copy of the *cta* gene on an *lt* variegation-inducing chromosome rearrangement (Wakimoto and Hearn 1990). We looked for variegation of *cta* in larval tissues using lt^{G10}/Df larvae, which have a single variegating copy of the *cta* gene (Wakimoto and Hearn 1990). We determined that *cta* was expressed in a variable fraction of the cells of the salivary gland (Figure 9, A and B). In

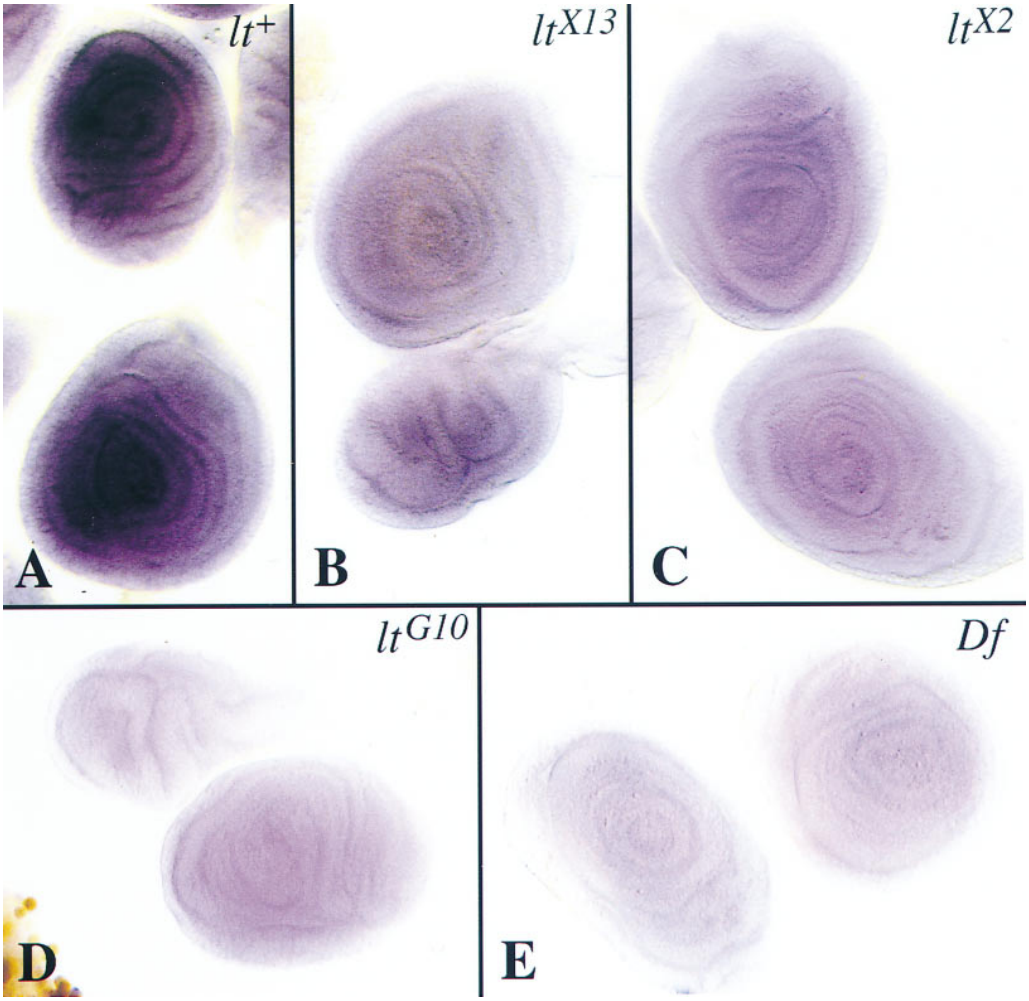

Figure 6.—Expression of *lt* in leg imaginal discs. *In situ* hybridization to *lt* mRNA of leg discs from larvae bearing a *lt*-variegating rearrangement showed a reduced, nonvariegated staining pattern. Typical staining levels are shown for leg discs from larvae bearing a *lt* deficiency chromosome heterozygous with the indicated *lt* allele, from a single experiment.

contrast, the *cta* gene appeared to be uniformly expressed in the cells of the imaginal discs. Figure 9 also illustrates the relative *cta* mRNA staining levels of cta^{+}/Df (C and F), lt^{G10}/Df (D and G), and $Df(2L)lt^{X120}/Df$ (E and H) leg imaginal discs (C–E) and eye-antennal imaginal discs (F–H). Analogous to results for *lt* expression, a reduced level of *cta* mRNA staining was observed in lt^{G10}/Df imaginal discs, as compared to wild-type discs.

DISCUSSION

This study explores tissue-specific and developmental changes in chromosome structure using PEV of the heterochromatic *lt* and *cta* genes as tools. The aims of this study were to determine if heterochromatic gene expression is variegated in all larval tissues and if rearrangements may show tissue-specific differences in the ability to induce variegation. Our analyses revealed that each of three chromosome rearrangements reduced *lt* expression in all tissues examined. Interestingly, the relative severity of variegation in the adult eye, the larval Malpighian tubules, and the larval salivary glands was consistent in these tissues for the three *lt*-variegating alleles and reflected their effects on viability. These results suggest that variegation-inducing rearrangements do not have tissue-specific effects on gene expression. However, we did discover a developmental specificity to *lt* variegation. For example, the imaginal eye-antennal disc showed a uniform, reduced level of *lt* mRNA at the third larval instar although the adult eye exhibited variegated, sometimes patchy, *lt* expression. This nonvariegated mRNA staining pattern was observed in all imaginal discs for all three *lt*-variegating alleles. Moreover, the level of *lt* mRNA detected in these discs was consistent with the severity of *lt* variegation observed in variegated tissues. A second heterochromatic gene exhibiting PEV, the *cta* gene, was similarly found to have a variegated pattern of expression in the salivary glands but reduced, nonvariegated mRNA levels in the imaginal discs.

Our observation that rearrangements can cause both a uniform reduction and a variegated pattern of expression for a single gene was unexpected. Previous phenotypic observations have been consistent with the classification of chromosome rearrangements into two categories by Lewis (1950): those involving only euchromatic breakpoints can exert stable effects on gene expression and others involving both heterochromatic

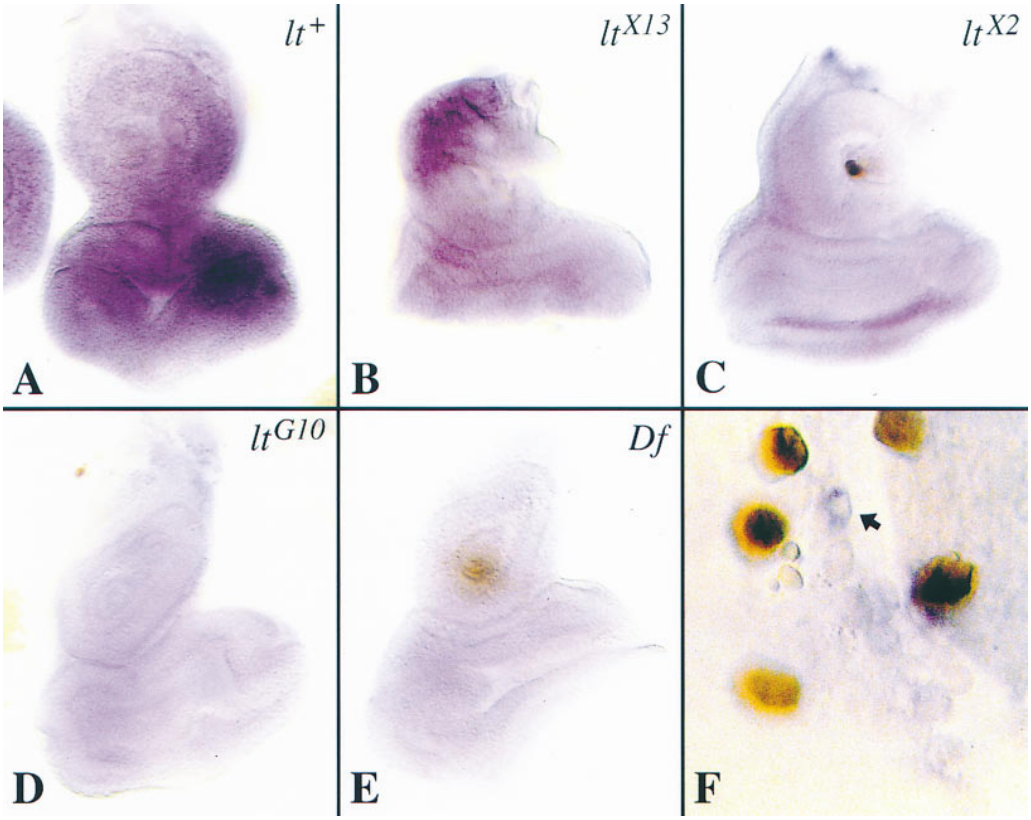

Figure 7.—Expression of *lt* in eye-antennal imaginal discs. (A–E) *In situ* hybridization to *lt* mRNA of eye-antennal discs from larvae bearing an *lt*-variegating rearrangement showed a reduced, nonvariegated staining pattern. Typical *in situ* hybridization staining levels are shown for eye-antennal discs derived from larvae bearing an *lt* deficiency chromosome heterozygous with the indicated *lt* allele, from a single experiment. As described in the text, variations in tissue thickness and folding of the tissue, especially for the eye-antennal discs, caused certain areas of the tissue to appear darker. All larvae had the *Bc* mutation, resulting in large dark brown melanotic crystal cells. These crystal cells were associated with most eye-antennal discs and are evident in C (center of upper antennal portion of the disc), E (center of antennal portion of the disc), and F. Strong staining for *lt* RNA was observed in a subset of the hemocytes and was most apparent against a background of weak staining, as was obtained for discs from lt^{X2}/Df and lt^{G10}/Df larvae. A high magnification view of hemocytes associated with a lt^{G10}/Df disc is shown in F. The *lt* staining pattern was typically cytoplasmic, but a nuclear dot was also observed for some cells (arrow).

and euchromatic breakpoints can exert variegated effects on gene expression. However, the present analyses of the effects of rearrangements with heterochromatic and euchromatic breakpoints on *lt* and *cta* expression illustrate that rearrangements can no longer be simply characterized as inducing either stable or variegated effects. For consistency, we retain the designation of "*lt*-variegating" to describe these rearrangements, even though the current study illustrates that the effect of this class of rearrangements was decreased nonvariegated expression in the imaginal discs. A greater understanding of the relationship of the two elicited phenotypes should be enlightening as to the mechanisms of chromatin-induced position effects.

Analyses of the effects of chromosome rearrangements on gene expression using *in situ* hybridization to RNA: We have used RNA *in situ* hybridization to examine *lt* expression in nonpigmented tissues. This approach monitors expression at the cellular level and has the added advantage of more accurately reflecting the transcriptional state of a gene compared to methods measuring its protein product or ultimate phenotype. Several factors can influence the concentration of specific cellular mRNAs, including the frequency of transcription initiation, the efficiency of RNA processing steps, and mRNA stability. While certain mutations and conditions can modulate these steps, the effects of variegation-inducing chromosome rearrangements are believed to be mediated through altered chromatin structure. We therefore think it most likely that we have assayed changes in transcription initiation or transcript elongation. For simplicity, the models presented below refer to transcription initiation, although they apply as well to synthesis of a full-length transcript.

We conclude from the nonvariegated imaginal disc staining pattern observed for lt^{var}/Df larvae that all imaginal cells transcribed the *lt* gene, but at a lower level than that of cells bearing the nonrearranged allele. An alternate possibility, which we do not favor, is that variegated expression of *lt* occurred in imaginal cells, but the *lt* mRNA was sufficiently stable to mask the variegation pattern. If so, every imaginal cell was either currently expressing *lt* or had inherited *lt* mRNA due to expression in a previous generation. The uniformity of staining makes this possibility unlikely because it would require that the combined amount of *lt* message was similar regardless of when and how long *lt* transcription was "on" versus "off" in each cell's lineage. At the least, we would expect to have observed the effect of the exponential dilution of *lt* RNA resulting from cell divi-

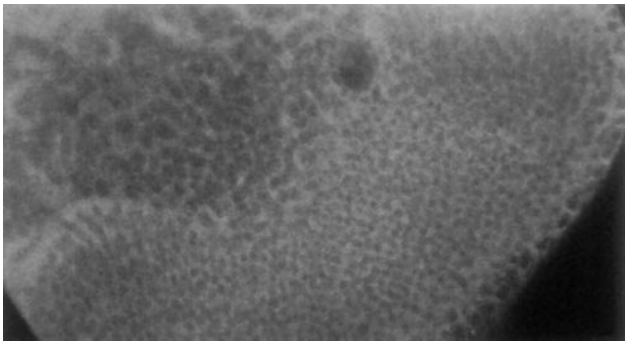

Figure 8.—Fluorescent detection of *lt* mRNA in eye-antennal imaginal discs from lt^{X2}/Df larvae. Uniform staining of eye-antennal discs was observed using *in situ* hybridization to *lt* mRNA and confocal microscopy. A representative 0.1-μm optical section from the lower eye portion of an eye-antennal imaginal disc is shown. The nuclei appear as black holes.

sion in a lineage in which *lt* is turned "off." Our staining methods were sufficiently sensitive to reveal a twofold difference in *lt* mRNA level (*e.g.*, compare lt^+/lt^+ and lt^+/Df imaginal discs in Figure 3, A and B), as would occur in the first generation following repression of *lt*. Smaller differences in *lt* RNA levels in the range between no *lt* expression and one copy lt^+ expression were also detectable (Figures 6, B–D and 7, B–D). Thus the absence of cell-to-cell variations in staining strongly suggests that transcription of the *lt* gene itself was not variegated. Moreover, it is difficult to conceive how altering the frequency of expressing cells could have given rise to different, but consistent, uniform mRNA levels for the three lt^{var} alleles. Thus, the possibility of variegated expression in the imaginal discs appears incompatible with our results.

Models for nonvariegated heterochromatic gene expression in imaginal discs: A decrease in *lt* cytoplasmic RNA levels could reflect either (1) a shorter period of a normal rate of transcription during the cell cycle, or (2) a decrease in rate with the duration unaffected. A position effect of the first type was observed for two human β-globin transgenic mouse lines, by Milot *et al.* (1996); only a fraction of the nuclei were transcribing the transgene at any given time, although all cells exhibited a reduced cytoplasmic RNA level. One model of this kind that could explain the rearrangement-induced decrease in *lt* and *cta* mRNA accumulation presupposes that the chromatin structure necessary for full transcriptional activity of heterochromatic genes is established during mitosis, and that subsequent DNA replication disrupts this structure. As a result of the chromosome rearrangement, the timing of replication of the heterochromatic domain could be shifted to an earlier point in the cell cycle, prematurely terminating active transcription. Consequently, the cytoplasmic RNA concentration would be inversely proportional to the precociousness of replication of the locus. In support of this model, studies in several systems indicate that there is a dramatic remodeling of chromatin components during mitosis (Aparicio and Gottschling 1994; Martinez-Balbas *et al.* 1995). Furthermore, heterochromatic portions of eukaryotic genomes are generally later replicating than euchromatic regions, and chromosome rearrangements with breakpoints within these two domains could conceivably alter replication patterns in diploid cells. Indeed, rearrangements which cause *lt* variegation do decrease the degree of DNA representation of the *lt* gene in polytene tissues (M. Hearn and B. Wakimoto, unpublished data; M. Howe and B. Wakimoto, unpublished data).

A second model is based on the idea of a decreased rate of transcription, to explain the reduction of *lt* and *cta* transcripts in imaginal tissues. It predicts that initiation or elongation of transcription is impeded as a result of disruption of normal heterochromatin formation in the vicinity of these heterochromatic genes. The consequence of chromosome rearrangements with breakpoints in the heterochromatin proximal to the *lt* gene and in the distal euchromatin, is the isolation of a subregion of heterochromatin including *lt* and other heterochromatic genes. This isolation reduces the variety and quantity of heterochromatin in the vicinity of the *lt* gene, and in this way might restrict its ability to associate with particular heterochromatic proteins. For example, the affinity of a heterochromatic region for particular proteins and/or its propensity to assume specific chromatin conformations may be proportional to the quantity of certain repetitive sequence elements. Subdividing the total amount of any one repetitive element could have a dramatic effect on the efficacy of that region to attract a protein and/or take on a particular chromatin structure. If this rearrangement-induced heterochromatin protein deficiency includes one or more heterochromatic proteins that are required indirectly as local chromatin morphogens or directly as transcription factors for *lt* transcription, then expression would be expected to be reduced. We favor this second model because it shares much in common, mechanistically, with the compartment model proposed to explain the variegated expression of heterochromatic genes (see below).

What determines whether a chromosome rearrangement causes variegated or reduced, uniform heterochromatic gene expression? We attribute the disparate effects of the *lt* variegation-inducing chromosome rearrangements, *i.e.*, mosaic expression in the adult eye, and larval Malpighian tubules and salivary glands, but nonvariegated reduced expression in imaginal tissues, to the differing developmental states of these cells. A diagram illustrating this model is shown in Figure 10. The imaginal disc cells are undifferentiated and are cycling or newly postmitotic. We propose that the heterochromatic factors required for *lt* transcription are abundant within these undifferentiated nuclei. However, the impaired ability of the displaced heterochromatic region to attract the appropriate quantity and variety of heterochro-

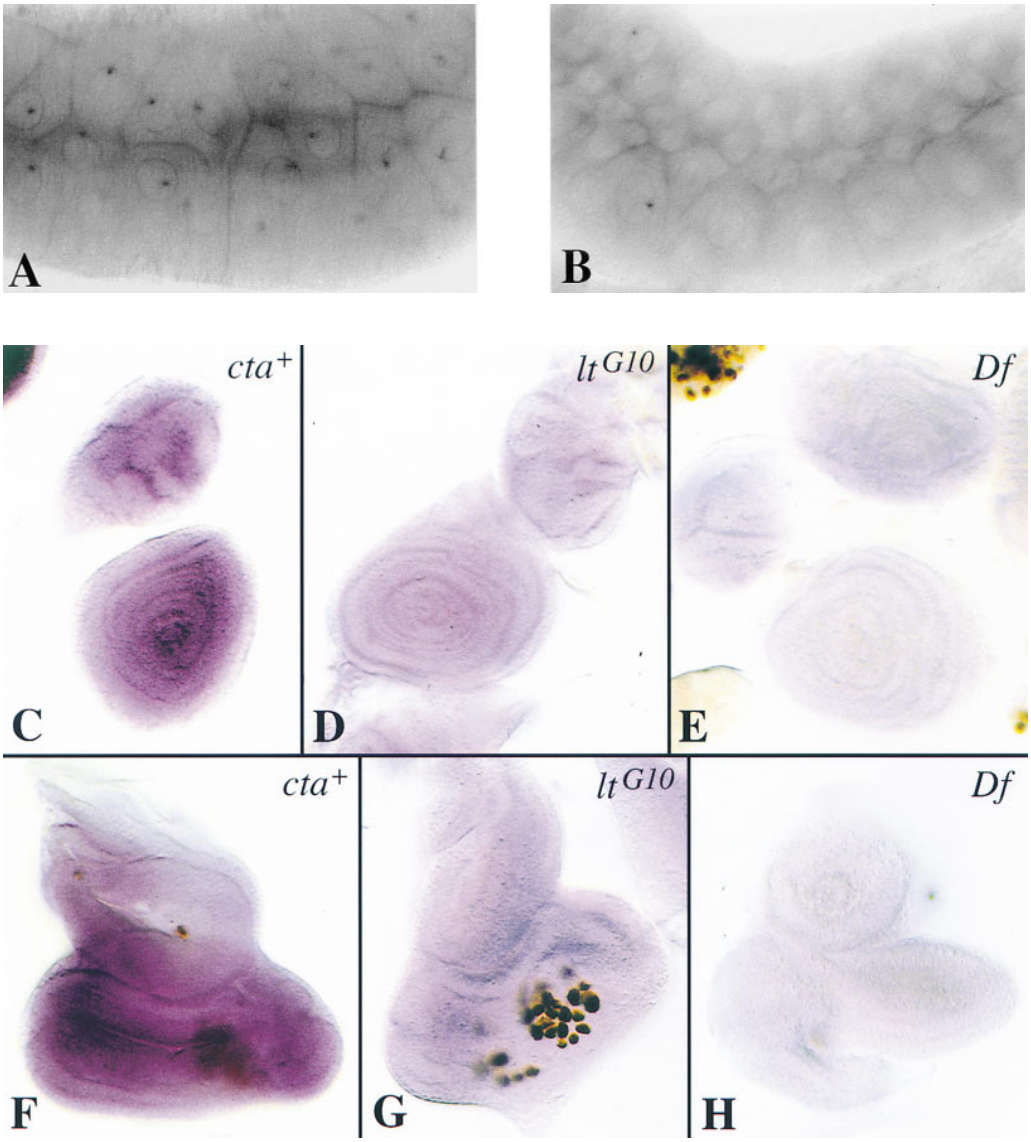

Figure 9.—Effects of the lt^{G10} rearrangement on *cta* expression, as detected by *in situ* hybridization. (A and B) Salivary glands from larvae bearing a *cta*-variegating rearrangement exhibited a mosaic staining pattern for *cta* RNA. A single focus of nuclear staining was detected throughout the salivary glands from cta^{+}/Df larvae (A), but in only a minority of cells of glands from lt^{G10}/Df larvae (B). (C–H) The imaginal discs derived from larvae bearing a *cta* deficiency chromosome heterozygous with the indicated *cta* allele. Uniform, cytoplasmic staining for *cta* mRNA was observed in leg discs (C–E) and eye-antennal discs (F–H), from a single experiment. The presence of dark brown melanotic crystal cells due to the *Bc* mutation can be seen in F–H.

matic proteins (as proposed above) results in reduced *lt* transcription. In the differentiated nucleus, the displaced subregion of heterochromatin is likewise compromised in its ability to attract heterochromatic proteins. However, we suggest that a restriction in the abundance of these factors (perhaps related to the cessation of mitotic chromosome condensation) and the establishment of chromosomal interactions accompany differentiation and result in cell-to-cell differences in expression state. In the nuclear context of limiting concentrations of heterochromatin proteins, the relative ability of the isolated heterochromatic region to compete with pericentric heterochromatin for these components might often be insufficient to support any transcription. However, in a subset of the cell population physical interactions between the displaced heterochromatic region and pericentric heterochromatin could ameliorate this situation and allow for full expression (the compartment model; Wakimoto and Hearn 1990). We propose that the transition of the imaginal cells into a postmitotic, differentiated state allows for the formation of associations between different heterochromatic domains that are absent or transient in dividing cells. These associations are postulated to be important for the exchange of heterochromatin proteins and/or induction of a heterochromatic state necessary for heterochromatic gene expression.

The correlation between the reduction of *lt* transcription in the imaginal discs and the severity of variegation in the eye, salivary gland, and Malpighian tubules for the lt^{G10}, lt^{X2}, and lt^{X13} alleles suggests a common factor influencing both phenotypes. According to our model, this correlation reflects the importance of the quantity of the displaced subregion of heterochromatin to heterochromatic gene expression throughout development. Cytological analyses of the *lt*-variegating rearrangements indicated that the *lt* gene-containing heterochromatic block was smaller for the lt^{G10} chromosome than

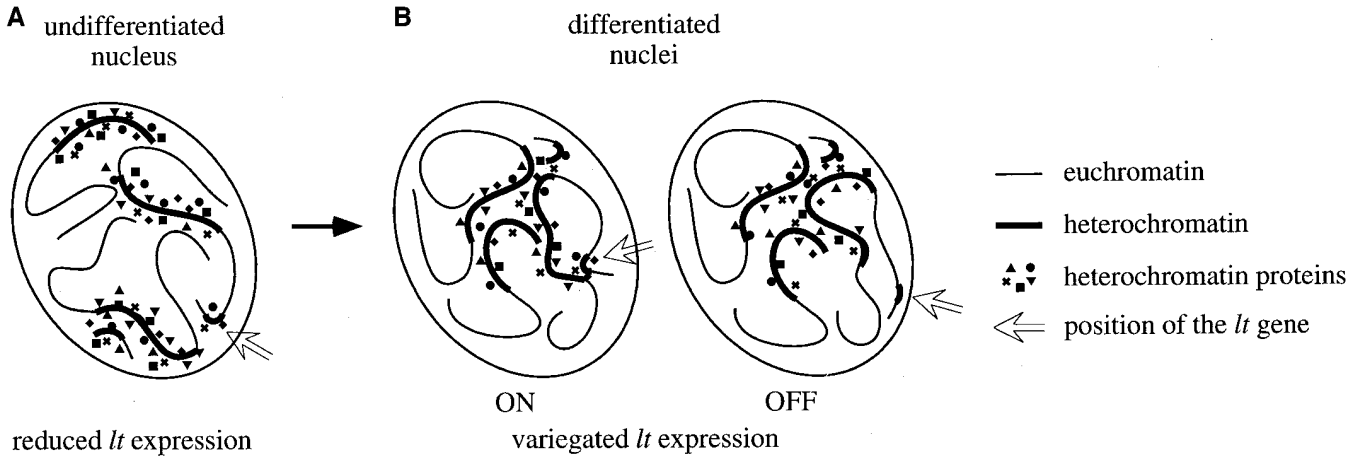

Figure 10.—A model to explain developmental differences in the effect of lt^{var} rearrangements on *lt* expression. Expression of the heterochromatic *lt* gene in both undifferentiated and differentiated cells is sensitive to the association of heterochromatin proteins with surrounding repetitive DNAs. Within the undifferentiated nucleus, heterochromatin proteins are abundant. When the *lt* gene is present on a nonrearranged chromosome, it has a full complement of heterochromatin proteins and is expressed normally. However, the isolation of a subregion of heterochromatin due to chromosome rearrangement, as shown in A, leads to a reduction in the association of heterochromatin protein(s) and reduced *lt* expression. Following differentiation (B), heterochromatic gene expression may be further compromised as a result of a decrease in the nuclear concentration of heterochromatin proteins. However, one or a few areas of high local concentration (compartments) of heterochromatin are formed by associations between regions of heterochromatin. Cells bearing an *lt*-variegating chromosome rearrangement either express the *lt* gene or do not express the *lt* gene, depending on whether the displaced heterochromatic region is located within a heterochromatin compartment.

for the lt^{X2} and lt^{X13} chromosomes (that were not distinguishable from each other cytologically), consistent with the more severe effect of lt^{G10} on gene expression (Wakimoto and Hearn 1990). We propose that in imaginal cells, the expression level of the translocated *lt* gene is reduced due to the decrease in quantity of associated heterochromatic factors, which in turn reflects the amount of surrounding heterochromatic DNA. When these cells differentiate, the magnitude of the displaced heterochromatin is also likely to influence its frequency of interaction with other heterochromatic compartments, and thus the severity of variegation.

The model we propose to explain the developmental change in the effects of chromosome rearrangements on heterochromatic gene expression bears similarity to the transvection effects model of Golic and Golic (1996). They proposed that the pairing ability of homologous chromosomes in mitotic cells was governed by cell cycle length such that homolog asynapsis induced by structural heterozygosity might be overcome given a long enough interphase. Our model that the interaction between displaced heterochromatin and pericentric regions is transient or absent in imaginal disc cells, but forms in a subset of differentiated cells, follows a similar rationale. However, we have yet to demonstrate these inferred heterochromatic associations and to show that they correlate with *lt* expression. Although a few studies on variegation of the euchromatic *brown* (*bw*) gene have examined the association of the bw^D heterochromatic insertion with pericentric heterochromatin, the cells examined do not express the *bw* gene (Talbert *et al.* 1994; Csink and Henikoff 1996; Dernburg *et al.* 1996). In the experiment most relevant to our studies of *lt* variegation, the bw^D-pericentric heterochromatin association was measured using squashed cell preparations derived from the eye-antennal imaginal disc (Dernburg *et al.* 1996). Only a low frequency of association was observed, perhaps reflecting the transient nature of the association in imaginal cells, or a mixed population of cell types in the preparation. Interestingly, the bw^D-pericentric heterochromatin association was not observed in embryonic cells (Dernburg *et al.* 1996), possibly attributable to their short cell cycle length.

The relationship between position effect variegation of euchromatic and heterochromatic genes: It should be informative to study the properties of variegating alleles of other genes in order to gain further insight into chromatin behavior during development. Expression analyses of variegating genes conducted at the cellular level in multiple tissues at different stages of development have been performed for only one euchromatic gene (Lu *et al.* 1996) and two heterochromatic genes (this study), and may not reveal the full range of phenotypic effects of rearrangements. The characterization of variegating alleles of additional euchromatic genes is especially important as housekeeping genes or genes that are constitutively expressed may not behave similarly to the inducible *hsp70-lacZ* gene. Preliminary analyses of a variegating allele of the euchromatic glycerol-3-phosphate dehydrogenase (*gpdh*) gene by *in situ* hybridization to RNA suggest that differences may exist. Larvae bearing the T(Y:2)D222 rearrangement that in-

duces *gpdh* variegation (R. MacIntyre, personal communication) showed reduced, uniform imaginal disc expression (K. S. Weiler, unpublished data). However, we cannot firmly conclude that *gpdh* variegation shows developmental specificity because of the absence of homozygous *gpdh* RNA null larvae to serve as a negative control for nonspecific staining. It may also be relevant that two adjacent euchromatic reporter genes present on a variegation-inducing rearrangement were observed to differ in the severity and pattern of variegation (Wines *et al.* 1996). However, these phenotypic differences could result from differences in the cell types compared and/or the transcriptional regulation of the two reporter genes.

The analyses of PEV of *hsp70-lacZ*, *lt*, and *cta* suggest that heterochromatin formation is more dynamic during development than would have been predicted based upon early studies. Our conclusion that variegation of the *lt* and *cta* genes is established during differentiation is consistent with the results of Lu *et al.* (1996) indicating that *hsp70-lacZ* variegation changes during eye differentiation. We postulate that a decrease in abundance of some heterochromatin proteins upon differentiation is in part responsible for the transition in *lt* expression from a uniform to a variegated pattern. This idea would similarly explain the relaxation in repression observed for *hsp70-lacZ* induction during the same time period.

Previous studies have shown that heterochromatic genes require a heterochromatic environment for their normal expression (Wakimoto and Hearn 1990; Eberl *et al.* 1993), and we propose that a failure to achieve an optimally heterochromatic state is responsible for the observed decreased expression of the *lt* gene in imaginal discs. However, this suboptimal heterochromatic state may be quite sufficient to have an antagonistic effect on euchromatic gene expression. Hence, the model we have proposed to explain the nonvariegated effect of chromosome rearrangements on heterochromatic gene expression in imaginal discs can accommodate the results of Lu *et al.* (1996) showing variegation of inducible *hsp70-lacZ* expression in these tissues. The mosaicism of *hsp70-lacZ* expression could reflect cell-to-cell differences in the association of one or more heterochromatin proteins at the site of the transposon insertion (*i.e.*, spreading), whereas heterochromatic gene expression could reflect the status of the entire heterochromatic domain. In support of this model, we have previously shown that the severity of variegation of the euchromatic *w* gene was not indicative of the quantity of adjacent heterochromatin (Howe *et al.* 1995). Rather, *w* variegation was sensitive to the nature of the juxtaposed repetitive DNA. Taken together, these studies reflect the diverse biological activities of heterochromatin and the fact that position effects exerted by rearrangements on euchromatic *vs.* heterochromatic genes are not strictly reciprocal phenomena.

We thank J. Eissenberg, S. Henikoff, R. Levis, R. Wright, and members of our laboratory for comments on the manuscript. We are grateful to G. Schubiger for the use of his microscope and for suggestions, and to D. Duncan for providing an *in situ* hybridization protocol for larval tissues. This work was supported by National Science Foundation grant MCB9506916 to B.T.W. and American Cancer Society postdoctoral grant PF-3821 to K.S.W.

LITERATURE CITED

Aparicio, O. M., and D. E. Gottschling, 1994 Overcoming telomeric silencing: a transactivator competes to establish gene expression in a cell cycle-dependent way. Genes Dev. **8(10):** 1133–1146.

Baker, W. K., 1963 Genetic control of pigment differentiation in somatic cells. Am. Zool. **3:** 57–69.

Becker, H. J., 1961 Untersuchungen zur Wirkung des Heterochromatins auf die Genmanifestierung bei *Drosophila melanogaster*. Verh. Dtsch. Zool. Ges. **1960:** 283–291.

Boyd, L., E. O'Toole and C. S. Thummel, 1991 Patterns of *E74A* RNA and protein expression at the onset of metamorphosis in *Drosophila*. Development **112:** 981–995.

Csink, A. K., and S. Henikoff, 1996 Genetic modification of heterochromatic association and nuclear organization in *Drosophila*. Nature **381:** 529–531.

Dernburg, A. F., K. W. Broman, J. C. Fung, W. F. Marshall, J. Philips *et al.*, 1996 Perturbation of nuclear architecture by long-distance chromosome interactions. Cell **85:** 745–759.

Devlin, R. H., B. B. Bingham and B. T. Wakimoto, 1990 The organization and expression of the *light* gene, a heterochromatic gene of *D. melanogaster*. Genetics **125:** 129–140.

Dorn, R., S. Heymann, R. Lindigkeit and G. Reuter, 1986 Suppressor mutations of position-effect variegation in *Drosophila melanogaster* affecting chromatin properties. Chromosoma **93:** 398–403.

Eberl, D., B. J. Duyf and A. H. Hilliker, 1993 The role of heterochromatin in the expression of a heterochromatic gene, the *rolled* gene of *Drosophila melanogaster*. Genetics **134:** 277–292.

Edgar, B. A., and P. H. O'Farrell, 1989 Genetic control of cell division patterns in the Drosophila embryo. Cell **57:** 177–187.

Fanti, L., M. Berloco and S. Pimpinelli, 1994 Carnitine suppression of position effect variegation in *Drosophila melanogaster*. Mol. Gen. Genet. **244:** 588–594.

Golic, M. M., and K. G. Golic, 1996 A quantitative measure of the mitotic pairing of alleles in *Drosophila melanogaster* and the influence of structural heterozygosity. Genetics **143:** 385–400.

Grigliatti, T., 1991 Position-effect variegation—an assay for nonhistone chromosomal proteins and chromatin assembly and modifying factors, pp. 588–625 in *Functional Organization of the Nucleus: A Laboratory Guide. Methods in Cell Biology, Vol. 35*, edited by B. A. Hamkalo and S. C. R. Elgin. Academic Press, San Diego.

Hessler, A. Y., 1958 V-type position effects at the *light* locus in *Drosophila melanogaster*. Genetics **43:** 395–403.

Howe, M., P. Dimitri, M. Berloco and B. T. Wakimoto, 1995 *Cis*-effects of heterochromatin on heterochromatic and euchromatic gene activity in *Drosophila melanogaster*. Genetics **140:** 1033–1045.

Janning, W., 1970 Bestimmung des heterochromatisierungsstadiums beim *white*-positionseffekt mittels rontgeninduzierter mitotischer rekombination in der augenanlage von *Drosophila melanogaster*. Mol. Gen. Genet. **107:** 128–149.

Kim, N., J. Kim, P. Dongkook, C. Rosen, D. Dorsett *et al.*, 1996 Structure and expression of wild-type and suppressible alleles of the Drosophila *purple* gene. Genetics **142:** 1157–1168.

Lewis, E. B., 1950 The phenomenon of position effect. Adv. Genet. **3:** 73–115.

Lu, B. Y., C. P. Bishop and J. C. Eissenberg, 1996 Developmental timing and tissue specificity of heterochromatin-mediated silencing. EMBO J. **15:** 1323–1332.

Martin, D. I. K., and E. Whitelaw, 1996 The vagaries of variegating transgenes. BioEssays **18:** 919–923.

Martinez-Balbas, M. A., A. Dey, S. K. Rabindran, K. Ozato and C. Wu, 1995 Displacement of sequence-specific transcription factors from mitotic chromatin. Cell **83:** 29–38.

Milán, M., S. Campuzano and A. García-Bellido, 1996 Cell cycling

and patterned cell proliferation in the wing primordium of Drosophila. Proc. Natl. Acad. Sci. USA **93:** 640–645.

Milot, E., J. Strouboulis, T. Trimborn, M. Wijgerde, E. De Boer *et al.*, 1996 Heterochromatin effects on the frequency and duration of LCR-mediated gene transcription. Cell **87:** 105–114.

Moore, G. D., D. A. Sinclair and T. A. Grigliatti, 1983 Histone gene multiplicity and position effect variegation in *Drosophila melanogaster*. Genetics **105:** 327–344.

Mottus, R., R. Reeves and T. A. Grigliatti, 1980 Butyrate suppression of position-effect variegation in *Drosophila melanogaster*. Mol. Gen. Genet. **178:** 465–469.

Muller, H. J., 1930 Types of visible variations induced by X-rays in Drosophila. J. Genet. **22:** 299–335.

O'Farrell, P. H., B. A. Edgar, D. Lakich and C. F. Lehner, 1989 Directing cell division during development. Science **246:** 635–640.

Parks, S., and E. Wieschaus, 1991 The Drosophila gastrulation gene *concertina* encodes a Gα-like protein. Cell **64:** 447–458.

Pillus, L., and M. Grunstein, 1995 Chromatin structure and epigenetic regulation in yeast, pp. 123–146 in *Chromatin Structure and Gene Expression*, edited by S. C. R. Elgin. IRL Press, New York.

Reuter, G., R. Dorn and H. J. Hoffmann, 1982 Butyrate sensitive suppressor of position effect variegation mutants in *Drosophila melanogaster*. Mol. Gen. Genet. **188:** 480–485.

Reuter, G., and P. Spierer, 1992 Position effect variegation and chromatin proteins. BioEssays **14:** 605–612.

Slobodyanyuk, S. Y., and O. L. Serov, 1987 Stage and organ specificity of the degree of variegation of the 6-phosphogluconate dehydrogenase gene in *Drosophila melanogaster*. Mol. Gen. Genet. **208:** 329–334.

Talbert, P. B., C. D. S. Leciel and S. Henikoff, 1994 Modification of the Drosophila heterochromatic mutation *brown-Dominant* by linkage alterations. Genetics **136:** 559–571.

Tautz, D., and C. Pfeifle, 1989 A nonradioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals translational control of the segmentation gene *hunchback*. Chromosoma **98:** 81–85.

Tobler, J. E., J. J. Yim, E. H. Grell and K. B. Jacobson, 1979 Developmental changes of sepiatpterin synthase activity associated with a variegated *purple* gene in *Drosophila melanogaster*. Biochem. Genet. **17:** 197–206.

Wakimoto, B. T., and M. G. Hearn, 1990 The effects of chromosome rearrangements on the expression of heterochromatic genes in *Chromosome 2L* of *D. melanogaster*. Genetics **125:** 141–154.

Wallrath, L., and S. C. R. Elgin, 1995 Position effect variegation in Drosophila is associated with an altered chromatin structure. Genes Dev. **9:** 1263–1277.

Weiler, K. S., and B. T. Wakimoto, 1995 Heterochromatin and gene expression in Drosophila. Annu. Rev. Genet. **29:** 577–605.

Wilson, C., H. J. Bellen and W. J. Gehring, 1990 Position effects on eukaryotic gene expression. Annu. Rev. Cell Biol **6:** 679–714.

Wines, D. R., P. B. Talbert, D. V. Clark and S. Henikoff, 1996 Introduction of a DNA methyltransferase into Drosophila to probe chromatin structure in vivo. Chromosoma **104:** 332–340.

Communicating editor: J. A. Birchler