polyhomeotic regulatory sequences induce developmental regulator-dependent variegation and targeted P-element insertions in Drosophila

Marie-Odile Fauvarque and Jean-Maurice Dura
Centre National de la Recherche Scientifique | CNRS URA 1134, Laboratoire d'Embryologie Moléculaire. Université Paris XI-Bât. 445, 91405 Orsay Cedex, France

Variegation of the miniwhite gene is observed in a euchromatic context in transformant lines that contain a P transposon including regulatory sequences of the polyhomeotic (ph) gene upstream of the resident miniwhite gene [Pph]. This variegated phenotype is not affected by most of the genetic modifiers of heterochromatic position-effect variegation (PEV) nor by removal of the Y chromosome. Interestingly, it is sensitive to ph and Polycomb (Pc) mutations, which are known to affect homeotic gene regulation. Regulatory DNA of ph can also mediate transvection of the miniwhite gene. This transvection is abolished in a ph but not in a zeste mutant background. In addition, Pph inserts preferentially in sites corresponding to PH/PC protein-binding sites as defined at the polytene chromosome level. These insertions induce an unusually high proportion of mutations in genes affecting homeotic gene regulation. In particular, one insertion is located within the tramtrack locus, which is thought to regulate fushi tarazu, an Ultrabithorax activator. We suggest that a multimeric complex containing PH and PC proteins, at a minimum, causes a local and clonally inherited heterochromatinization, which maintains the repressed state of transcription of the homeotic genes.

[Key Words: Homeotic gene regulation; local heterochromatinization; Polycomb group genes; target gene identification; transvection]

Received March 5, 1993; revised version accepted June 3, 1993.

In Drosophila, the segmental identity of the body depends on the spatially restricted expression of the homeotic genes of two complexes: Antennapedia [ANT-C] and bithorax [BX-C] [Lewis 1978; Kaufman et al. 1980; Sanchez-Herrero et al. 1985; for reviews, see Akam 1987; Sanchez-Herrero et al. 1985; Ingham and Martinez-Arias 1986; Paro and Stortion 1989]. The maintenance of this specific pattern depends on two groups of trans-regulator genes: the trithorax group [trx-G] and the Polycomb group [Pc-G] [Lewis 1978; Struhl 1981; Duncan 1982; Jürgens 1985; Kennison and Tamkun 1988; for reviews, see Ingham 1985; Paro and Stortion 1989]. The emergence of the specific expression pattern of the homeotic genes in the blastoderm requires the activity of early-acting segmentation genes [Duncan 1986; Ingham and Martinez-Arias 1986; Irish et al. 1989]. The maintenance of this specific pattern depends on two groups of trans-regulator genes: the trithorax group [trx-G] and the Polycomb group [Pc-G] [Lewis 1978; Struhl 1981; Duncan 1982; Jürgens 1985; Kennison and Tamkun 1988; for reviews, see Ingham 1985; Paro 1990; Kennison and Tamkun 1992], which maintain the active and inactive state, respectively, of homeotic gene transcription during the rest of development [Struhl and Akam 1985; Wedeen et al. 1986; Dura and Ingham 1988; Huang and Dawid 1990; Jones and Gelbart 1990; Mazo et al. 1990; McKeon and Brock 1991; Simon et al. 1992; Tamkun et al. 1992]. The polyhomeotic (ph) locus has been defined as a member of the Pc-G because it exhibits the posteriorly homeotic transformations characteristic of Pc-G mutant phenotypes [Dura et al. 1985, 1987, 1988]. Members of the Pc-G act synergistically on homeotic gene transcription—double or triple mutant combinations showing more dramatic homeotic transformations than the simple addition of each mutant effect [Jürgens 1985]. Mutations in the trx-G antagonize and suppress mutations in the Pc-G [Ingham 1985], and most members of the trx-G were screened according to this criterion [Kennison and Tamkun 1988].

The two proteins PH and PC were found to be associated with ~100 common discrete sites on the polytene chromosomes. The PH/PC target sites include the two homeotic complexes but also several loci corresponding to trx-G and Pc-G genes [Zink and Paro 1989; Zink et al. 1991; DeCamillis et al. 1992]. In particular, the locus 2D, where ph is located, is included in PH/PC target sites, indicating that ph expression may be regulated by PH and PC proteins. PH and PC proteins were shown to bind chromatin in a DNA-specific manner, although neither PH nor PC was shown to bind directly to naked DNA [Zink et al. 1991; DeCamillis et al. 1992]. As they coprecipitate with ~10–15 other proteins, suggesting that they act in a multimeric complex, it is likely that at least one of the other components binds to the DNA [Franke...
et al. 1992]. The discovery of a homologous domain, the "chromodomain," between PC and a nonhistone heterochromatin-associated protein HP1 [Paro and Hogness 1991] has provided significant advances in the understanding of the molecular mechanism by which members of the Pc-G may act on their targets. Removal of this chromodomain from PC abolishes its attachment to the chromatin [Messmer et al. 1992]. Pc-G proteins may act by inducing a local heterochromatin-like structure ensuring the transcriptional repression of their target gene, particularly the homeotic genes, which would thus be maintained in a clonally repressed state [Paro 1990]. However, no genetic or molecular evidence that members of the Pc-G are effectively able to induce a local heterochromatinization has yet been provided.

In this paper we describe variegation of the miniwhite gene in a euchromatic context in lines that contain a P transposon, including regulatory sequences of ph upstream of the miniwhite gene [P[ph]]. Normally, variegated eyes are observed when a chromosomal rearrangement places the euchromatic white gene next to heterochromatin, and this phenomenon is called position–effect variegation (PEV) [for reviews, see Eisenberg 1989; Henikoff 1990; Tartof and Bremer 1990; Reuter and Spierer 1992]. In the case of PEV, the clonal white expression results from the spreading of the heterochromatic domain to include, in some cells, the white gene, thereby inhibiting its expression. Once established, the heterochromatin is then maintained as such in the daughter cells leading to a clonal expression of white in the eye. Here, we show that the variegated phenotype of the P[ph] lines is not the result of PEV because the insertions are located in euchromatic areas; moreover, this variegation is neither modified by most genetic modifiers of PEV nor by removal of the Y chromosome, which greatly enhances PEV. Interestingly, the variegated phenotype of the P[ph] transgenic lines depends on a normal dosage of PH and PC proteins. This suggests that Pc-G genes may act by inducing a local heterochromatinization. We also found that ph regulatory DNA can mediate transvection of the white gene. This transvection is abolished in ph but not in a zeste mutant background.

The genetic and molecular identification of all the components of the Pc-G and trx-G is crucial for the understanding of their mode of action. As a number of these loci correspond to PH/PC target sites, the identification of PH/PC target genes may provide a means to find other members of the Pc-G and trx-G, putative new homeotic genes, and potential new classes of target genes. Here, we describe the utilization of the P[ph] transposable element as a putative powerful method for the screening of PH/PC target genes. This element inserts preferentially into sites that correspond to PH/PC-binding sites as detected at the polytene chromosome level. Some of them are within genes that act in homeotic gene regulation. Thus, the P[ph] transposable element may appear to be useful for both the identification of PH/PC target loci and the understanding of the molecular mechanism by which homeotic genes are regulated.

Results

ph regulatory DNA induces variegation of the white gene in transgenic lines

In an effort to study the regulation of ph expression throughout development, we made transgenic constructs containing different regulatory regions of the ph locus driving the lacZ gene [P[ph]; Fig. 1]. In one set of experiments [418 and 419 constructs], the transforming vector contained ph regulatory DNA upstream of the miniwhite gene driven by its own promoter. In a high proportion of P[418] transgenic lines (50/130 = 38%), differing from either transformation or transposition, we observed variegation of white expression. One of six 419 transformed lines also showed this phenotype. Two types of variegated phenotype can be distinguished [Fig. 2]; the patterned phenotype, in which all of the individuals exhibit a reproducible patterned white expression, and the mottled phenotype, in which expression of white appears randomly, very likely in a clonally inherited manner. For most strains, a mixture of the two phenotypes is observed. Variegation of the white gene following transformation or transposition is normally a rare event [Hazelrigg et al. 1984; Levis et al. 1985]. No more than 1% of anomalous expression of white was detected when hundreds of lines carrying a P-lacW insertion were made [Dura et al. 1993]. We injected the pCaSpeR-AUG-β-gal vector [Thummel et al. 1988] without any ph sequences, and we obtained 14 independent lines. None of them showed any sign of white gene variegation. Therefore, our working hypothesis is that ph regulatory sequences are directly responsible for the variegated expression of the miniwhite gene.

The variegated phenotype induced by ph regulatory sequences is not attributable to PEV

Variegated expression of a euchromatic gene is seen when a chromosomal rearrangement places it next to heterochromatin (for recent reviews, see Eisenberg 1989; Tartof et al. 1989; Henikoff 1990; Reuter and Spierer 1992). This PEV is dependent on genetic elements like the Y chromosome and suppressor and enhancer loci. We investigated whether or not the ph-induced variegation was similar to PEV. First, for the insertions inducing variegated eyes that we mapped (n = 24), all but one were in euchromatic, rather than in heterochromatic, areas of the polytene chromosomes [for details, see Table 2, below]. Second, for seven strains that we have tested [T71.1, T22.31, T3, T13.4, T30, T42.1, T72.2], the absence of the Y chromosome has no effect on XO male eye color. In contrast, as expected, enhancement of PEV [in w<sup>-</sup> males] attributable to absence of Y was observed. Third, we tested the effect of 22 suppressors and 9 enhancers of variegation [listed in Materials and methods] on the T3 line, and 4 suppressors and 6 enhancers on three other 418 lines [T7.1, T53.3, and T30]. These four 418 lines exhibit variegation visible in heterozygous flies. In almost all cases, no effect of these modifiers of PEV was detectable in 418 lines. However,
Figure 1. (A) Schematic representation of ph proximal unit and of fragments 418, 419, 800, and 804 containing various ph sequences. Distances are indicated in kb. Solid boxes represent regions homologous with the distal ph unit. Restriction sites are abbreviated as follows: (X) XbaI; (P) PstI; (E) EcoRI; (K) KpnI. (B) Position of 418 and 419 fragments in transposon P[ph]. Arrows indicate the direction of transcription.

Figure 2. 418 variegated phenotype at 25°C. (A) 17.1/Y, patterned expression in which all individuals exhibit a reproducible white expression; (B) T15.2/T15.2, mottled expression in which expression of white appears randomly, probably in a clonally inherited manner; (C) T3/T3, mixture of patterned and mottled white expression.

Ph regulatory DNA-induced variegation is modified by developmental regulators

PC and PH proteins are part of a multimeric complex that binds chromatin at ~100 sites in the polytene chromosomes (Franke et al. 1992). One of these sites corresponds to the ph locus itself (Zink and Paro 1989; Decamillis et al. 1992). Therefore, we tested the effect of ph and Pc mutations on ph-induced variegated eye phenotype. All the variegated strains tested, except one, showed modification of the eye color in ph and/or Pc mutant backgrounds (Table 1). When the change occurs, it is always darker, that is, variegation is suppressed in a
Local heterochromatinization and targeted P insertion

**Figure 3.** DREV is enhanced while PEV is suppressed at higher temperatures. (A) w^coe, 17°C; (B) w^coe, 25°C; (C) T30, 17°C; (D) T30, 25°C.

*ph^-/+ or ph^-/Y context and it is lighter, that is, variegation is enhanced in a Pc^-/+ context. As for the 418 lines, the variegating 419 line (Table 1) is sensitive to both ph and Pc mutations. Interestingly, in three 419 nonvariegating lines (of a total of five), a variegated phenotype is induced in a Pc^- background. In contrast to ph and Pc mutations, no effect was detected in fifteen 418 variegated lines in a trx^3 mutant background [lines tested: T3, T13.5, T26, T30, T33.1, T24, T40, T42.1, T43, T44, T53.3, T63, T66.1, T69.3, T72.1, T72.2].

Transgenic lines for a P[w^+] element containing the regulatory region of en (P[en]) also show patterned eyes in a high proportion of cases [Kassis et al. 1991]. The product of en is ectopically expressed in a ph mutant background [Dura and Ingham 1988], and the en locus (48A) corresponds to a PH/PC protein-binding site. This suggests that PH could also modify the variegated phenotype induced by en regulatory sequences. We observed that the eye color becomes darker in ph^{a10} males in three P[en] lines, G6, F3, and SR1-c, but not in a fourth, F4 [Kassis et al. 1991]. However, the suppression of the variegated phenotype by ph is less marked than in the case of P[ph] transgenic lines. These results indicate that the variegation observed in the case of both P[ph] and P[en] transgenic lines is probably induced by some common developmental factors, particularly the ph product. There is no visible effect of Pc^K mutation on these four P[en] lines.

Because ph regulatory DNA-induced variegation is not attributable to PEV but is modified by developmental regulators like ph and Pc, we propose calling this new kind of variegation developmental regulator effect variegation [DREV].

**lacZ gene expression also variegates**

None of the four series of transgenic lines, 418, 419, 800, and 804 [see Fig. 1], contains sufficient ph regulatory sequences to initiate a wild-type ph pattern of lacZ expression in embryos as it is described for ph transcripts [Deatrick 1993]. However, in six variegating lines tested (12.2, 31, T3, T26, T30, T40, T66.1), we observed variegated expression of the lacZ gene in imaginal discs (Fig. 4), which suggests that ph regulatory sequences induce variegation to both the left and right.

**ph regulatory DNA induces miniwhite gene transvection**

The miniwhite gene is not fully functional, and the resulting eye color is dependent on gene dosage: homozygous flies ordinarily have darker eyes than heterozygous flies [Pirrotta 1988]. For most of the variegated 418 lines (31/44), homozygous individuals have lighter eye color than do heterozygous flies (Fig. 5, cf. T3/+ and T3/T3). This phenomenon is very likely to be similar to the transvection effect induced by en regulatory DNA on
### Table 1. Variegated phenotype and effect of *ph* and *Pc* mutation on various 418 and 419 lines at 25°C

| Insert  | Hemizygous | Heterozygous | Homozygous | *ph*<sup>410</sup> | *Pc*<sup>K</sup> |
|---------|------------|--------------|------------|-----------------|----------------|
| 418     |            |              |            |                 |               |
| 17.1    | pat        | pat          | pat        | L               | D             | L             |
| T2.2;3t | —          | —            | mot        | L               | =             | L             |
| T13.4   | mot        | —            | mot        | L               | D             | L             |
| T26     | —          | —            | mot        | L               | D             | L             |
| T40     | pat/mot    | —            | pat/mot    | L               | D             | L             |
| T43     | —          | —            | pat/mot    | L               | D             | L             |
| T63     | pat        | pat          | —          | =               |               |               |
| T72.1   | —          | —            | pat/mot    | D               | D             | L             |
| 11.2.2  | pat        | —            | —          | =               |               |               |
| 133.1   | pat        | —            | —          | =               |               |               |
| T3      | pat/mot    | pat/mot      | —          | =               |               |               |
| T13.5   | pat/mot    | pat/mot      | —          | =               |               |               |
| T15.2   | —          | —            | mot        | =               |               |               |
| T30     | mot        | —            | —          | =               |               |               |
| T33.1.2 | —          | —            | mot        | =               |               |               |
| T33.1.5 | —          | —            | mot        | =               |               |               |
| T33.1.24j | pat/mot | lethal       | —          | =               |               |               |
| T42.1   | pat/mot    | —            | pat/mot    | D               | D             | L             |
| T44     | pat        | —            | lethal     | =               |               |               |
| T53.3   | pat        | —            | pat        | D               | D             | L             |
| T66.1   | pat/mot    | —            | pat/mot    | —               | D             | =             |
| T69.3   | —          | —            | pat        | =               | D             | =             |
| T72.2   | mot        | —            | mot        | L               | L             | =             |
| 419     |            |              |            |                 |               |
| 419/6.1 | pat        | —            | —          | D               | D             | L             |
| 419/5.1 | —          | —            | —          | D               | =             | V             |
| 419/8.1 | —          | —            | —          | D               | =             | V             |
| 419/19.1| —          | —            | —          | D               | =             | V             |

Hemizygous mutants are described in the cases of X-linked insertion. Homozygous mutants have lighter (L) or darker (D) eyes than heterozygous. Heterozygote insert lines have darker (D) eyes in *phi*<sup>+</sup> or *ph/Y* background and lighter (L) in *Pc/+* background, or they are identical (=). Variegation appears in three of five originally nonvariegating 419 lines in *Pc/+* background (V); pat] Patterned white expression; (mot) mottled white expression.

white expression [Kassis et al. 1991]. No transvection effect was detected in the 14 *P[pcSpeR-AUG-β-gal]* control lines. Transheterozygotes for two transVecting *P[phi]* inserts located at the same site on the polytene chromosome, also show transvection of the mini*white* gene. This was demonstrated for the two X-linked lines, *T72.1* and *T63*, mapping at 12D, and for the three lines *T15.2*, *T33.3*, and *T69.3* (all combinations tested) mapping at 35C. The eye pattern of the lines that have the same insertion site are almost identical. Thus, the patterned aspect of the eye probably depends on the genomic sequences surrounding the transposon.

We tested the effect of *ph*<sup>410</sup> on the transvection of 10 transVecting autosomal 418 lines (*T33.1*, *T3*, *T13.5*, *T15.2*, *T30*, *T42.1*, *T53.3*, *T66.1*, *T69.3*, *T72.2*). When a *phi*<sup>410</sup> mutant background, either in homozygous or hemizygous condition, is introduced in such transVecting lines, the transvection effect is completely abolished: In *phi*<sup>410</sup> context, individuals homozygous for the insert have darker eyes, actually wild type, than the heterozygous flies [Fig. 5, cf. *ph*<sup>410</sup>/Y;*T3*<sup>+</sup> and *ph*<sup>410</sup>/Y;*T3*/T3]. This suggests that PH protein plays an active role in causing white expression to be repressed in 418 homozygous lines, possibly via *ph* regulatory DNA sequences. Transvection of the *white* gene was also described for some of the *P[en]* insertions described above. At least for one line, *F3*, transvection is abolished in *phi* mutant background. In contrast to the *ph* mutation, null mutations of either *Pc* (*Pc*<sup>K</sup>) or *trx* [*Df(3R)red-P93*] genes have no effect on *phi*-mediated transvection as tested on five X-linked transVecting lines (*T13.4*, *T26*, *T40*, *T43*, *T72.1*).

---

**Figure 4.** Variegated expression of the lacZ gene in two wing imaginal discs from the *T40* line.
Local heterochromatinization and targeted \( P \) insertion

The \textit{zeste} gene has been implicated in mediating transvection for many different genes (Gans 1953; Gelbart and Wu 1982; Wu and Goldberg 1989, Pirrota 1990). The effects of \( z^u \), an amorphic allele of \textit{zeste}, was tested on seven X-linked 418 lines \((17.1, T2.2;31, T13.4, T26, T40, T43, T72.1)\). Only the transvection of the \( T2.2;31 \) line is suppressed in a \( z^u \) background. No effect of \( z^u \) on the eye color of the other six strains was detected. These data suggest that the Z protein is not an essential component for the interaction mediated by the \( ph \) sequence.

A number of \( P/ph \) elements insert preferentially within genes involved in homeotic gene regulation

Because chromosomal localization was of primary importance to correctly interpret the observed DREV phenomenon, we localized 19 independent variegating insertions on polytene chromosomes (Table 2). Surprisingly >50\% (11) are localized in the same polytene chromosomal section as a PH/PC protein-binding site (Zink and Paro 1989; DeCamillis et al. 1992), which suggests that \( P/ph \) inserts nonrandomly and preferentially into PH/PC target genes. Another proof of the directed nature of the insertion events is that two locations (12D and 35C) had two and three independent inserts, respectively.

However, cytogenetic localization is rather imprecise at the molecular level because two apparently coincident cytological binding sites might be separated by as much as 200 kb. To examine the hypothesis of \( P/ph \) preferential insertion in PH/PC targets, we looked at the nature of \( P/ph \)-induced mutations. Because a high number of PH/PC chromosomal binding sites are located where \( Pc-G \) and \( trx-G \) loci map (Zink and Paro 1989; DeCamillis et al. 1992), the \( Pc-G \) and \( trx-G \) genes are probably part of a genetically regulated network. Consequently, a certain number of the \( P/ph \) inserts may be within \( Pc-G \) and \( trx-G \) gene, mutants which enhance and suppress \( ph \) and \( Pc \) phenotypes, respectively. We tested all of the 418 lines for which insertion had produced a lethal or visible phenotype (16 lines) and lethal excisions of two X-linked lines \((17.1 \) and \( T2.2;31)\). We have also tested, in homozygous condition, 10 randomly chosen, variegating viable 418 lines. Each of these lines was tested for enhancement or suppression of the homeotic transformations induced by \( ph \) and/or \( Pc \) mutations (for details, see Materials and methods). Among 130 \( P/ph \) lines, we analyzed 28 lines and found 3 \( Pc-G \) members and 1 \( trx-G \) member (the results are summarized in Table 3). This proportion is at least 100 times higher than that which can be obtained with a similar \( P \) element not carrying \( ph \) regulatory sequences (for more details, see Discussion). We note that two of these four lines do not variegate, which suggests that preferential insertion can occur independently of the variegated phenotype. Therefore it seems clear that an unusually high proportion of genes acting in...
Table 2. Cytogenetic localization of variegating insertions on polytene chromosomes

| Insertions | Chromosome | In situ localization | PH/PC protein-binding sites |
|------------|------------|---------------------|-----------------------------|
| T43        | 1          | 5D                  | 5D                          |
| T40        | 1          | 9D                  |                             |
| T7.1       | 1          | 12D                 | 12D                         |
| T6.3       | 1          | 12D                 | 12D                         |
| T26        | 1          | 12E                 |                             |
| T2.2;31    | 1          | 14B                 | 14B                         |
| T13.4      | 1          | 20D                 |                             |
| T44        | 2          | 24D                 |                             |
| T42.1      | 2          | 28B                 |                             |
| T53.3      | 2          | 35C                 | 35C                         |
| T15.1      | 2          | 35C                 | 35C                         |
| T69.3      | 2          | 35C                 | 35C                         |
| T41.2b     | 2          | 35CD                | 35C                         |
| T13.5      | 2          | 36BC                | 36AC                        |
| T33.1      | 2          | 38F                 | 38F                         |
| T12.1.2    | 2          | 56F                 | 56F                         |
| T33.24d    | 2          | 60B                 |                             |
| T15.2      | 3          | 65CD                | 65D                         |
| T3         | 3          | 65F                 |                             |
| T66.1      | 3          | 84DE                | 84D                         |
| T33.1;2    | 3          | 84F                 | 84F                         |
| T30        | 3          | 86CD                | 86C                         |
| T33.1;5    | 3          | 88C                 |                             |
| T11.1b     | 3          | 88D                 |                             |
| 804-41.5b  | 3          | 100D                | 100D                        |

Table 3. Effect of four P[ph] insertions on homeotic transformations induced by ph and/or Pc mutations

| P[ph] insertion | MT to Pro leg | MS to Pro leg | Antenna to leg |
|----------------|---------------|---------------|--------------|
| 2.2;31/±       | —             | —             | 1.53**       |
| control        | —             | —             | 1.15         |
| T11.1/±        | 2.67*         | 2.82**        | 1.33*        |
| control        | 2.00*         | 1.55          | 1.13         |
| T41.2/±        | 1.50**        | 1.00**        | 1.09**       |
| control        | 2.47          | 1.50          | 1.27         |
| T66.1/T66.1    | 2.83**        | —             | —            |
| control        | 1.71          | —             | —            |

Transformations were scored as described in Materials and methods. Each number represents the mean class of 50 legs or antennae. The probability \( P \) that the two populations containing or not containing the insertion are identical was calculated with the Chi\(^2\) test. (MS) Mesothoracic; (Pro) prothoracic; (MT) metathoracic. (*) \( P < 0.05 \); (**) \( P < 0.001 \).

In \( ph^{41} / y \) context.

In \( Pc^{+/+} \) context.

In \( ph^{41}/y ; Pc^{+/+} \) context.

An 804 insertion locates in the tramtrack gene

Transgenic lines 800 and 804 (Fig. 1A) do not show any sign of variegation, probably because modified transposons \( P[800] \) and \( P[804] \) contain the HSP70 promoter upstream of the white gene [Kuhn et al. 1988]. This does not exclude the possibility that preferential insertion may be obtained with these two transposons. Therefore, we mapped one of the nineteen 800 and 804 insertions on polytene chromosomes because it is embryonic lethal. Interestingly this insertion 804-41.5 is located at 100D in a PH/PC protein-binding site. Moreover, we demonstrated by Southern analysis that it maps within the \( tramtrack (ttk) \) locus. In two different genomic digestions probed with the p69 form of \( ttk \) cDNA (Read and Manley 1992), a new band appears in the 804-41.5 line as compared with the two controls: the strain \( w^{1118} \), which was transformed with \( ph \) and \( Pc \) mutations, and the strain \( w^{1118} / y ; Pc^{+/+} \) (Fig. 1B). The gene \( ttk \) was isolated as a gene encoding a \( fushi tarazu \) (ftz) promoter-binding protein [Harrison and Travers 1990; Brown et al. 1991]. It also codes for a related protein that binds to the promoter of another pair-rule gene: the \( even-skipped \) (eve) gene (Read and Manley 1992). This 804-41.5 insertion, which has probably occurred via a preferential insertion, provides the first mutant allele for \( ttk \) as no mutant has been described previously. The embryonic lethal allele that we obtained exhibits strong cuticular defects [Fig. 7]: Heads do not develop properly; thoracic and abdominal segments seem to be present, but ventral denticle bands are significantly reduced; spiracles and filzk6rpers are still detectable; and dorsal closure is not always achieved. Thus, \( ttk \) product appears to be an essential factor for the development of the embryo.

Figure 6. Insertion 804-41.5 is located in the \( ttk \) gene. Genomic DNAs of \( w^{1118} \) (A), 804-41.5/± (B), and 804-29.1/± (C) were digested by \( EcoRI \) or \( BamHI \) and probed with the cDNA p69 of \( ttk \). Arrows indicate the appearance of a new band.
Local heterochromatinization and targeted P insertion

Figure 7. Cuticular phenotype of ttk embryos. Ventral is at the bottom and anterior is at the left. The head fails to develop properly. Ventral denticle bands are quasi-absent.

Discussion

PH and PC proteins modify the variegated phenotype mediated by ph regulatory sequences

ph and Pc are members of the Pc-G genes that maintain the repressed state of the homeotic genes during embryogenesis of Drosophila. The PC protein has a region—the chromodomain—which shows 65% similarity to the nonhistone heterochromatin-associated protein HP1 [Paro and Hogness 1991]. The HP1 protein is encoded by Su(var)205, a suppressor of PEV [Eissenberg et al. 1992]. Before this homology was known, Locke et al. (1988) proposed a model, based solely on genetic experiments, suggesting a mechanistic link between the Pc-G gene family and the modifiers of PEV. This chromodomain is responsible for the binding of PC protein to chromatin [Messmer et al. 1992]. On the basis of this homology, a model has been proposed where the repressed state of homeotic genes is maintained and clonally inherited by means of a local induction of a heterochromatin-like structure [Paro 1990]. This local DNA compaction could be induced by a multimeric complex composed of different proteins of the Pc-G, including at least PH and PC proteins [Franke et al. 1992]. It was never demonstrated, however, that Pc or other members of the Pc-G are effectively able to induce a local heterochromatinization. Genomic insertion of a modified P element carrying ph regulatory sequences upstream of the miniwhite gene [P[ph]] induces variegated eyes in 38% of the transgenic lines. This particular variegated phenotype is not the result of insertion into heterochromatin and is generally not modified by genetic modifiers of PEV. In contrast, mutations in either ph or Pc genes, respectively, suppress and enhance this variegated phenotype. We propose, therefore, to call this new variegation, DREV. We have also observed that ph mutation partially suppresses variegation of P[en] transgenic lines [Kassis et al. 1991], suggesting that this variegation is the result of events similar to those in the variegation induced by ph sequences.

To explain the random expression of the miniwhite gene, we hypothesized that certain proteins are able to induce a local heterochromatinization at the level of the transposon. The variegation phenotype normally occurs in the near vicinity of heterochromatin, thus, by analogy, DNA compaction at the level of the transposon may result in a structure similar to that of heterochromatin. Therefore, we will now use the term of local heterochromatinization to describe this phenomenon. Do PH and PC proteins take part in this local heterochromatinization? A 50% reduction of these proteins is able to modify and even induce, in the case of PC, a variegated phenotype, and both have been shown to bind the chromatin of their target loci in a DNA sequence-dependent manner [Zink et al. 1991; DeCamillis et al. 1992]. As PH and PC are known to bind in 2D, where the ph locus lies [Zink and Paro 1989; DeCamillis et al. 1992], we suggest that the presence of ph regulatory sequences allows the binding of PH and PC proteins and, perhaps, other members of the Pc-G. This binding may generate a local heterochromatinization leading to the observed variegated phenotype (Fig. 8). Although we cannot exclude that the effect of PH and PC proteins is not direct, it is clear that this variegated miniwhite gene expression depends on ph and Pc product. This supports very strongly the idea that these two proteins act by inducing a local heterochromatinization. DREV should provide a new and useful tool to study the mechanism by which homeotic genes are regulated through local heterochromatinization.

Figure 8 Hypothetical model for creating a local heterochromatinization leading to the variegating phenotype. Binding of a PH/PC multimeric complex on ph regulatory sequences would generate a local heterochromatinization, including the white gene, as well as the lacZ gene, and therefore lead to the observed variegated phenotype. Open boxes indicate compacted and, therefore, inactive chromatin.
Fauvarque and Dura

DREV and PEV may be the result of analogous chromatin constitution

DREV is not only influenced by developmental regulators like PH and PC proteins, for example, increasing the temperature induces an enhancement of DREV while inducing a suppression of PEV (Tartof and Bremer 1990). This may indicate that the chromatin organization that induces DREV is formed by multimeric units, the concentration of which depends on a mass-action model (Locke et al. 1988).

DREV is also influenced by some modifiers of PEV (5 of 31 tested), but the effects of these modifiers are not always the same as for PEV (3 of 5). These results suggest that some proteins implicated in PEV may also be implicated in the DREV phenomenon. For example, in the case of DREV, once the heterochromatinization signal is given by developmental regulators, the effective DNA compaction is likely to depend on certain structural proteins implicated in the physical conformation of the heterochromatin. If some of these proteins are identical to those implicated in the formation of centromeric heterochromatin, that would indicate that the local heterochromatin made in DREV has, effectively, a physical constitution similar to the heterochromatin. However, the fact that DREV is influenced in a different way by temperature and by some modifiers of PEV, and not at all by removing the Y chromosome, provides evidence that physical components operating in DREV and PEV contain some structural differences.

P[ph] transposon may insert preferentially into PH/PC target genes

Approximately 100 common target loci have been defined on polytene chromosomes for the two proteins PH and PC. Neither PH nor PC has been shown to bind DNA directly, but both proteins coprecipitate in the same multimeric complex that is thought to mediate the observed DNA binding (Franke et al. 1992). Known loci are members of PH/PC targets, especially the two homoeotic gene complexes BX-C and ANT-C, and many Pc-G and trx-G genes (Zink and Paro 1989; DeCamillis et al. 1992). As assayed by chromosomal in situ hybridization experiments, the insertion of the P[ph] transposon at the chromosomal level frequently occurs at PH/PC protein-binding sites. Thus, it was possible that it occurred in Pc- or trx-G genes. We found three enhancers and one suppressor of ph and/or Pc mutations. These 4 loci were isolated from 28 transgenic lines chosen non-randomly from a pool of 130 418 lines. This exceptionally high rate of mutation induced by the insertion of the P[ph] transposon, in genes implicated in homeotic gene regulation, is to be compared with other results that we obtained: First, among 50 lines carrying one P[w] lethal insertion, none was found to interact with ph^{410}. Second, we screened 10,000 chromosomes carrying at least one P[w] insertion and found four suppressor genes, and no enhancers of ph^{410} (M.-O. Fauvarque and J.-M. Dura, unpubl.).

This illustrates particularly well the advantage of using the P[ph] element for finding genes that interact with ph. Thus, preferential insertions may provide a powerful method for identifying PH/PC target genes. In support of this idea, we obtained a ttk mutant allele, the first described to our knowledge, indicating that ttk is likely to be a PH/PC target gene. In addition, the presence of the transposon greatly facilitates further genetic analysis and molecular cloning.

Preferential insertion in the en region (Hama et al. 1990) and in genes exhibiting a stripe expression pattern (Kassis et al. 1992) was observed with transposons carrying en regulatory sequences [P[en]]. We suggest that regulatory proteins bring together transposons carrying ph or en regulatory sequences and the endogenous target gene sequences into which preferential insertion occurs. In this model the regulatory proteins common to the transposon and the target gene must be present in the germ line of flies in which transposition occurs. This is the case, at least in the female germ line, for both ph and Pc protein products [Paro and Zink 1992, M. DeCamillis and H. Brock, pers. comm.]. PH and PC proteins regulate ph expression as well as en expression (Busturia and Morata 1988; Dura and Ingham 1988, Moazed and O’Farrell 1992; this study). Therefore, PH and PC are good candidates for mediating the preferential insertion of the two transposons. This would explain the overlapping of preferential insertion sites for the two transposons P[ph] and P[en], illustrated by the high number of insertions in 35C [3/130 for P[ph] and 2/49 for P[en]]. However, not only PH and PC may mediate preferential insertion but any other protein of the specific multimeric complex that is supposed to bind ph (or en) regulatory sequences. All of the preferential insertions of the two transposons should not, then, be identical. Moreover, the Tll1.1 insertion that enhances ph phenotype is not located at a known PH/PC target site. We suggest that protein(s) other than PH/PC may mediate preferential insertion into a member of the Pc-G. Another possible explanation is that some target genes of PH/PC are not detectable, or only weakly, on salivary gland polytene chromosomes.

Regulation of the ph gene by the PH/PC multimeric complex

Contained among the 100 PH/PC-binding sites is the ph locus (Zink and Paro 1989; DeCamillis et al. 1992). However, it has not been demonstrated that PH and PC effectively regulate the expression of ph. On the contrary, no effect of Pc mutations on ph expression in embryos is observed [Franke et al. 1992]. However, our results suggest that PH and PC are regulators of ph later in development, as they are able to modify miniwhite gene expression when close to ph regulatory sequences. Furthermore, PH and PC are good candidates for mediating preferential insertion into PH/PC target genes by binding to ph sequences.

PH and PC have opposite effects on miniwhite gene expression, and by extension they may have opposite effects on ph expression. Pc may be an activator of ph,
whereas ph may be a repressor of its own expression. However, if ph is regulated by Pc, mutation of Pc will provoke a change in the amount of the ph product. Thus, the effect observed in Pc− context not only reflects the diminution of the Pc product but also a change in the amount of ph product (and other Pc-regulated genes). Because our results strongly suggest that ph regulates itself, conclusions about the mechanism of ph regulation by Pc are not immediately straightforward. It would not be surprising, however, that Pc acts positively on ph transcription because Pc and ph act synergistically on homeotic gene regulation (Dura et al. 1985). If Pc were to repress ph transcription, then Pc mutations would have a suppressor effect on homeotic transformation induced by the ph mutation. In this scheme, Pc (and ph) should act positively on the transcription on those Pc-G genes in which mutations act synergistically with Pc (and ph) in homeotic gene regulation.

PH acts on the ph regulatory DNA-mediated transvection effect

Homologous chromosomes are intimately paired in somatic cells of insects. The transvection effect originally defined by Lewis [1954] (for a recent review, see Wu and Golberg 1989) suggests a functional role for somatic pairing. In most of the variegating 418 lines, homozygous individuals have lighter eye color than heterozygous ones. This phenomenon can be classified as a transvection effect because according to the current definition, it is an “allelic interaction that shows dependence on chromosomal pairing or allele proximity” (Judd 1988). Although the phenomenon that we observe is similar to that mediated by the zeste gene at the white locus, the Z protein was not found to be an essential component of this interaction. Here, we showed that PH protein acts on the transvection effect induced by ph regulatory sequences. In a ph mutant background, the transvection effect is completely abolished while ph mutation does not affect the transvection of white induced by the z1 mutation (data not shown). We note that a transvection effect of the same kind mediated by a fragment of en regulatory DNA has been described recently (Kassis et al. 1991). At least in one case, the same suppression of transvection was seen in a ph mutant background. We suggest that the normal function of this transvection is similar and may be mediated by the same kind of proteins in both cases. This is the first description of a transvection role for the PH protein. This function does not seem to be shared by the PC protein because no effect of Pc mutation on ph-mediated transvection was observed. Thus, PH and PC proteins may act in a different way within the PH/PC multimeric complex. We propose that PH protein has a tendency to aggregate. This can be the case for other components of the multimeric complex as well. This aggregation will result in more complex forms when two copies of the transgene are in proximity to one another and, therefore, will strengthen repression. Thus, the transvection effect may represent a mechanism mediating the regulatory effect of PH.

Local heterochromatinization and targeted P insertion

Local heterochromatinization, preferential insertions, and transvection may all be explained by a tendency of the proteins that compose the multimeric complex to aggregate. The name “agregulate” has recently been proposed for such regulating complex (Jacob 1993). This provides a simple explanation of how the repression of homeotic genes is maintained during successive cell divisions: As soon as the newly formed DNA strand is synthesized, a complete multimeric complex will form under the influence of that part that remains on the old strand. This will ensure a clonal maintenance of the repressed state of determination.

Materials and methods

Fly strains and culture

All strains were maintained on standard culture medium at 17°C, 25°C, or 28°C. All variants used are described in Lindsley and Zimm (1992), except for the amorphic PcE allele, which is described in Zink and Paro (1989).

Vector and constructs

418 and 419 fragments were cloned in the P-element vector pCaSpeR-AUG–ß-gal (Thummel et al. 1988) in the orientation shown in Figure 1. 800 and 804 fragments were cloned in the P element vector pW–ATG–lac2 (Kuhn et al. 1988). Positions of restriction sites used for constructs are given in parentheses as referred to in Deatrich et al. (1991): 418, 2.9-kb fragment XbaI(3467)–PstI(6272), 419, 4.0-kb fragment EcoRI(1397)–Kpnl(5329), 800, 4.9-kb fragment EcoRI(1367)–PstI(6272); and 804, 8.8-kb fragment, which results from a partial digestion with PstI, EcoRI(1397)–PstI(10177 or 10309).

Generation of transgenic lines

P-element-mediated transformation was carried out essentially as described by Rubin and Spradling (1982). w118 embryos were injected with 150 mg/ml of helper plasmid [pUCHaPA2-3 (D. Rio, pers. comm.)] and 300 mg/ml of construct. The construct 418 was injected into a ry500Sb P[ry+Δ2-3](99B)/TM6,Ubx stock containing a stable source of transposase (Robertson et al. 1988). Transformants were identified on the basis of their eye color and established as homozygous or as balanced heterozygous stocks. New transgenic lines were generated by mobilization of P-element construct 418, using the stable source of transposase Δ2-3.

Strains that show interacting effects with ph and/or Pc mutants were outcrossed for eight generations with a w118 strain and were retested.

Modifiers of PEV tested with 418 lines

Twenty-two suppressors and nine enhancers of PEV were kindly provided by G. Reuter and J. Gauss (pers. comm.). Suppressors were Su(var)2(1)01, Su(var)2(5)05, Su(var)2(1)301, Su(var)2b601, Su(var)2(4)01, Su(var)2(5)01, Su(var)2(5)02, Su(var)2(5)03, Su(var)2b301, Su(var)2b4801, Su(var)2b204, Su(var)2b701, Su(var)2b801, Su(var)2(2)01, Su(var)2(1)001, Su(var)3(3)16, Su(var)3(1)03, Su(var)3c1101, Su(var)3c902, Su(var)3(1)002, Su(var)3(1)041, and Su(var)3(1)111. Enhancers were E-915, E-183/31, E-56/9, E-90, E-129/1, E-166/7, E-102/1, E-104, and E-(3)105. For all of the enhancers and for four suppressors [Su(var)2(1)01, Su(var)2b204, Su(var)3(1)03, Su(var)3(1)002], we determined that
they produced the expected effect on males carrying the w\textsuperscript{exco} allele. Only the Su-\textit{var}(2)/\textit{t(1)os} locus did not suppress the variegated phenotype of the w\textsuperscript{exco} allele. The effect on eye color is observed in males, issued from the cross between males carrying a modifier of PEV and females carrying the 41B insertion.

\textbf{Analysis of β-gal expression}

Embryos (0–12 hr) were stained with antibodies against β-galactosidase as described by Ingham and Martinez-Arias [1986]. Staining of imaginal discs was performed essentially as described in Ashburner [1989].

\textbf{Interaction with ph and Pc: modification of homeotic phenotypes}

The partial transformation of second and third legs to first legs in \textit{ph\textsuperscript{1118}}/Y or in \textit{Pc\textsuperscript{K/+}} males results in the appearance of sex comb teeth on the second and third legs of males where they are not normally found. These transformations are scored as follows: (1) In the case of \textit{ph\textsuperscript{1118}} males, third legs are ranked into three classes depending on the number of extra teeth. First class, 0–2 teeth, second class, 3–5 teeth, third class, more than 5 teeth. (2) In the case of \textit{Pc\textsuperscript{K/+}} males, second legs are ranked as for transformation 1. The partial transformation of antennae to legs in females \textit{ph\textsuperscript{1118}}/\textit{w\textsuperscript{1118}Pc\textsuperscript{K/+}} results in deformation of existing segments and the appearance of leg segments on the antennae. This transformation (3) is scored as follows: transformed antennae are ranked into two classes. First class, deformation more or less marked with no more than one new leg structure; second class, appearance of more than one new leg segment.

\textbf{Preparation of chromosome spreads and in situ localization of transposon on polytene chromosomes}

\textit{Drosophila} polytene chromosome spreads were prepared from salivary glands of third-instar larvae, which were grown at 17°C. Salivary glands were dissected in Ringer’s solution (7.5 grams of NaCl, 0.35 grams of KCl, 0.21 grams of CaCl\textsubscript{2} in 1 liter of sterile H\textsubscript{2}O). For fixation, three pairs of glands were transferred into a droplet of 45% acetic acid on a coverslip (20 mm\textsuperscript{2}) for 5 min. The coverslip was picked up with a poly-L-lysine-coated slide. (Coating of slides: Clean slides were dipped into a 0.1% poly-L-lysine solution [Sigma] diluted 5× in H\textsubscript{2}O, and air dried.) The coverslip was moved slightly with a pencil, and tapped extensively for at least 1 min with the eraser of a pencil without moving the coverslip. The slide was turned over onto blotting paper, and thumb pressure was applied to the preparation. The coverslip was flipped off with a razor blade after freezing in liquid nitrogen. Slides were immersed immediately into ethanol/acetic acid (3 : 1) for at least 10 min, dehydrated in 100% ethanol for 10 min, and air-dried. Slides can be kept for 2 months before pretreatment. Chromosomes were heat treated in 2× SSC at 68°C for 30 min. Slides were then dipped in 2× SSC at room temperature for 2 min. Chromosomes were acetylated for 8 min in freshly made 1.0 M triethanolamine (pH 8.0)/0.125% and acetic anhydride [vol/vol]. Slides were washed for 4× for 4 min each in 2× SSC at room temperature and dehydrated 2× for 5 min each in 70% ethanol and 5 min in 95% ethanol, and air-dried. Chromosomes were denatured in freshly prepared 0.07 N NaOH at room temperature for 3 min. Slides were washed 3× for 5 min each in 2× SSC at room temperature and dehydrated and air-dried as described above. The slides must be used for hybridization within 24 hr after pretreatment. Construction 41B was used as probe. Labeling was performed with the Random priming kit from Boehringer, using 1 μl of BiodUTP as labeled nucleotide (1 mM biotin-16-dUTP, Boehringer), and 500 ng of linearized DNA in a total volume of 20 μl. The reaction was stopped with 25 mM EDTA [final concentration]. DNA was precipitated with 2 volumes of ethanol in the presence of tRNA (2 mg/ml) and NaOAc (0.3 M) for 2 hr at −20°C. After centrifugation, the pellet was washed with 70% ethanol and resuspended in 150 μl of buffer Hbio [600 mM NaCl], 1× Denhardt’s, 50 mM NaPO\textsubscript{4} [pH 7.2], and 5 mM MgCl\textsubscript{2} conserved at −20°C. The probe can be conserved several months at −20°C. Twenty microliters of the probe, which was denatured and vortexed, was applied per slide. For all incubation steps, slides were kept in a humid atmosphere with a coverslip placed over the chromosomes. Hybridization was performed for 12–16 hr at 58°C with coverslips sealed with rubber cement. After hybridization, coverslips were removed and slides were washed for 3× for 20 min each in 2× SSC at 53°C. Slides were placed in a moist chamber. Coverslips were removed and slides were washed at room temperature for 1× for 2 min each in PBS [prepared from a 10× stock: 1.3 M NaCl, 0.07 M Na\textsubscript{2}HPO\textsubscript{4}, 0.3 M NaH\textsubscript{2}PO\textsubscript{4}], 1× for 2 min each in PBS/0.1% Triton-X-100 and 1× for 5 min each in PBS. Slides were air-dried and 100 μl of 1 : 200 diluted Detec-tI-Hrp [Kit Enzob EBP 820-1], in dilution buffer, was applied per slide. Slides were incubated for 30 min at room temperature in a moist chamber. Coverslips were removed and slides were washed at room temperature for 5 min in 2× SSC, for 5 min in PBS/0.1% Triton-X 100 and for 5 min in PBS. Slides were air-dried and 100 μl of the substrate mixture was applied per slide and the drop was covered with a coverslip. The substrate mixture was repared as follows: 3 μl of 30% H\textsubscript{2}O\textsubscript{2} was added to 1 ml of a solution of diaminobenzidine tetrahydrochloride (DAB) 0.5 grams/liter in PBS just before utilization.] After 8 min of reaction, coverslips were removed and slides were washed in D\textsubscript{2}O for 2× for 3 min each. (Caution: DAB is a powerful carcinogen. Work in the hood and use bleach to inactivate the DAB.) Chromosomes were stained for 30 sec in a solution of Giemsa [Merck 1 : 20 dilution in 10 mM sodium phosphate buffer at pH 6.8] and washed immediately for 30 sec in distilled water and air-dried. If necessary, staining time can be longer. Preparations were mounted in Euparal.

\textbf{Acknowledgments}

We thank Kathy Matthews, at the Indiana \textit{Drosophila} stock center, Renato Paro, Gunter Reuter, and Janos Gausz for providing stocks used in this study. We are particularly indebted to Ruth Griffin Shea for her valuable comments on the manuscript. Part of this work was performed at the Centre de Géné-tique Moléculaire/C.N.R.S at Gif sur Yvette, France. This work was supported by grants from the C.N.R.S. [ATIPE no. 7], from the Association pour le Recherche sur le Cancer and from the Ministère de l’Education Nationale (to M.O.F.).

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

\textbf{Note added in proof}

Since this paper was accepted for publication, two mutations (\textit{tkk}\textsuperscript{1} and \textit{tkk}\textsuperscript{101}) of the \textit{ttk} gene have been described [W.-C. Xiong and C. Montell 1993. \textit{Genes & Dev.} 7: 1085–1096]. The \textit{P}-element mutant we describe here will therefore be referred to as \textit{ttk}\textsuperscript{804}.
Fauvarque and Dura

...mic position on the expression of transduced copies of the white gene of Drosophila. Science 229: 558–561.
Lewis, E.B. 1954. The theory and application of a new method of detecting chromosomal rearrangements in Drosophila melanogaster. Am. Nat. 88: 225–239.
---. 1978. A gene complex controlling segmentation in Drosophila. Nature 276: 565–570.
Lindsley, D.L. and G.G. Zimm. 1980. A general overview of genetic transformation in Drosophila. Genetics 105: 1–16.
Mazo, A.M., D.H. Huang, B.A. Mozer, and I.B. Dawid. 1990. The trithorax gene, a trans-acting regulator of the bithorax complex in Drosophila, encodes a protein with zinc-binding domains. Proc. Natl. Acad. Sci. 87: 2112–2116.
McGinnis, W. and R. Krumlauf. 1992. Homeobox genes and axial patterning. Cell 68: 283–302.
McKee, J. and H.W. Brock. 1991. Interactions of the Polycomb group of genes with homeotic loci of Drosophila. Wilhelm Roux’s Arch. Dev. Biol. 199: 387–396.
Messmer, S., A. Franke, and R. Paro. 1992. Analysis of the functional role of the Polycomb chromo domain in Drosophila melanogaster. Genes & Dev. 6: 1241–1254.
Moazed, D. and P.H. O’Farrell. 1992. Maintenance of the engrailed expression pattern by Polycomb group genes in Drosophila. Development 116: 805–810.
Paro, R. 1990. Imprinting a determined state into the chromatin of Drosophila. Trends Genet. 6: 416–421.
Paro, R. and D.S. Hogness. 1991. The Polycomb protein shares a homologous domain with a heterochromatin-associated protein in Drosophila. Proc. Natl. Acad. Sci. 88: 263–267.
Paro, R. and B. Zink. 1992. The Polycomb gene is differentially regulated during oogenesis and embryogenesis of Drosophila melanogaster. Mech. Dev. 40: 37–46.
Pirotta, V. 1988. Vectors for P-mediated transformation in Drosophila. In Vectors: A survey of molecular cloning vectors and their uses (ed. R.L. Rodriguez and D.T. Denhardt), pp. 437–456. Butterworths, Boston, MA.
---. 1990. Transvection and long-distance gene regulation. BioEssays 12: 409–414.
Pirotta, V., S. Bickel, and C. Mariani. 1988. Developmental expression of the Drosophila zeste gene and localization of zeste protein on polytene chromosomes. Genes & Dev. 2: 1839–1850.
Read, D. and J.L. Manley. 1992. Alternately spliced transcripts of the Drosophila tramtrack gene encode zinc finger proteins with distinct DNA binding specificities. EMBO J. 11: 1035–1044.
Reuter, G. and P. Spierer. 1992. Position effect variegation and chromatin proteins. BioEssays 4: 605–612.
Robertson, H.M., C.R. Preston, R.W. Phillips, D.M. Johnson-Schitz, W.K. Benz, and W.R. Engels. 1988. A stable genomic source of P element transposase in Drosophila melanogaster. Genetics 118: 461–470.
Rubin, G.M. and A.C. Spradling. 1982. Genetic transformation of Drosophila with transposable element vectors. Science 218: 348–353.
Sanchez-Herrero, E., I. Vernos, R. Marco, and G. Morata. 1985. Genetic organization of Drosophila bithorax complex. Nature 313: 108–113.
Simon, J., A. Chiang, and W. Bender. 1992. Ten different Polycomb group genes are required for spatial control of the abdA and AbdB homeotic products. Development 114: 493–505.
Struhl, G. 1981. A gene product required for correct initiation of segmental determination in Drosophila. Nature 293: 36–41.
Struhl, G. and M. Akam. 1985. Altered distribution of Ultrabithorax transcripts in extra sex combs mutant embryos of Drosophila. EMBO J. 4: 3259–3264.
Tanak, J.W., R. Deuring, M.F. Scott, M. Kissinger, A.M. Pattatucci, T.C. Kaufman, and J.A. Kennison. 1992. brahma: A regulator of Drosophila homeotic genes structurally related to yeast transcriptional activators. Cell 68: 561–572.
Tartof, K.D. and M. Bremer. 1990. Mechanisms for the construction and developmental control of heterochromatin formation and imprinted chromosome domains. Development (Suppl.): 35–45.
Tartof, K.D., C. Bishop, M. Jones, C.A. Hobbs, and J. Locke. 1989. Towards an understanding of position effect variegation. Dev. Genet. 10: 162–176.
Thummel, C.S., A.M. Boulet, and H.D. Lipschitz. 1988. Vectors for Drosophila P-element-mediated transformation and tissue culture transfection. Gene 74: 445–456.
Weeden, C., K. Harding, and M. Levine. 1986. Spatial regulation of Antennapedia and bithorax gene expression by the Polycomb locus in Drosophila. Cell 44: 739–748.
Wu, C.-T. and M.L. Goldberg. 1989. The Drosophila zeste gene and transvection. Trends Genet. 5: 189–194.
Zink, B. and R. Paro. 1989. In vivo binding pattern of a trans-regulator of the homeotic genes in Drosophila melanogaster. Nature 337: 468–471.
Zink, B., Y. Engström, W.J. Gehring, and R. Paro. 1991. Direct interactions of the Polycomb protein with Antennapedia regulatory sequences in polytene chromosomes of Drosophila melanogaster. EMBO J. 10: 153–162.
polyhomeotic regulatory sequences induce developmental regulator-dependent variegation and targeted P-element insertions in Drosophila.

M O Fauvarque and J M Dura

*Genes Dev.* 1993, 7:
Access the most recent version at doi:10.1101/gad.7.8.1508