The 35UZ transposon of *Drosophila melanogaster* reveals differences in maintenance of transcriptional control between embryonic and larval stages

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ABSTRACT The *D. melanogaster* transposon P[35UZ] contains a lacZ reporter gene fused to 35 kb of *Ubx* upstream sequences which drive a *Ubx*-like expression in embryos and in metathoracic imaginal discs. Transposition of P[35UZ] followed by analysis of different lines in wild-type and mutant backgrounds allowed us to analyze the interplay between *Ubx* regulatory elements, including the Polycomb response element (PRE), located inside the transposon and cis-acting regulatory elements, located outside. We found that all lines show a *Ubx*-like β-galactosidase expression pattern in the embryo, but proximity to strong imaginal enhancers can change this pattern drastically. These data illustrate how maintenance of gene expression depends on the chromosomal environment and on dynamic interactions between all developmentally regulated enhancers located close to a promoter under PcG control.

KEY WORDS: silencing maintenance, developmental enhancers, PREs, polycomb group genes, *Ubx*

Maintenance of the expression domains of many selectors, as the *Hox* genes, depends on the negative trans-regulators coded by the Polycomb group of genes (PcG). These proteins form multimeric complexes which bind to cis-regulatory Polycomb response elements (PRE) in the genes to be silenced (reviews: Paro, 1995; Pirrotta, 1997, 1998). PcG mediated repression is thought to induce a chromatin structure whereinto proteins are allowed access according to shape and size (McCall and Bender, 1996). This phenomenon has been extensively studied with transposons containing a PRE, a reporter gene and a transcription activator binding element such as the GAL4 binding sequence UAS. Silencing of such a construct can be overcome by an excess of the transcription activator GAL4 (Zink and Paro, 1995). During embryogenesis, reversal is consistently obtained by a single burst of GAL4 production from a hs-GAL4 construct, whereas during the larval stages one burst of the activator apparently cannot permanently revert PRE-mediated gene repression (Cavalli and Paro, 1998), suggesting that it might show increased stability during the longer interphases of larval development.

Here, we have attempted a complementary approach to study factors that affect gene expression patterns in larvae, by analyzing a lacZ reporter gene controlled by regulatory sequences of the *Ultrabithorax* (*Ubx*) gene at different chromosomal locations and in different genetic backgrounds. Endogenous *Ubx* is expressed in the embryonic epidermis from PSS to PS13, with a strong peak in PS6, and in larvae in the posterior meso- and metathoracic disc compartments (review: Irvine et al., 1991). The two *Ubx* regulatory regions both contain embryonic control elements, imaginal enhancers and PREs (Fig. 1A) (review: Pirrotta et al., 1995). Most upstream embryonic control elements are contained in the 35 kb of *Ubx* sequences in transposon P[35UZ] (Fig. 1B) which drives a *Ubx*-like lacZ expression in embryos and in metathoracic discs (Irvine et al., 1991). This pattern due solely to upstream sequences mimics a bona fide *Ubx* in embryos and in larval PS6 (White and Wilcox, 1985; Little et al., 1990).

To assay the influence of different flanking sequences on P[35UZ], we examined lines bearing P[35UZ] at various chromosomal sites. Three transgenic lines, 35UZ-1 to -3, were generated by Irvine et al. (1991), and we recovered further 35UZ lines by mobilizing P[35UZ] from 35UZ-3. Southern blot analyses showed that six new lines (called the T lines) had a single insertion. Five

Abbreviations used in this paper: β-gal, beta-galactosidase; PcG, Polycomb group; *ph*, polyhomeotic gene; PRE, Polycomb response element; *Ubx*, *Ultrabithorax* gene; wt, wild type.

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contain an intact P[35UZ] transposon and one shows a small deletion in the 5' P-element sequences.

In embryos, all nine 35UZ lines revealed an epidermal pattern of β-galactosidase (β-gal) activity similar to wild-type (wt) Ubx expression (Fig. 2A) (Irvine et al., 1991), with occasional faint ectopic lacZ expression anterior to PS6 in late embryos. Strong ectopic β-gal activity was detected only in T17 embryos. In P[35UZ]/+ embryos mutant for an amorphic allele of the PcG gene polyhomeotic (ph), lacZ expression was found in parasegments anterior to PS5 in all nine 35UZ lines (Fig. 2B), showing that P[35UZ] lacZ expression in embryos is basically controlled by PcG regulation.

β-gal profiles were next determined in third instar larvae. Six lines showed the ‘35UZ pattern’, a metathoracic disc pattern similar to Ubx expression (Fig. 3A), sometimes associated with a few ectopic spots on notum primordia (Table 1). Two lines showed different and reproducible disc staining patterns. Irvine and Wieschaus (1994) previously reported that P[35UZ] at 78A in line 35UZ-1 has induced an allele of the fringe (fng) gene, which encodes a signaling molecule involved in cell interactions during development, and that 35UZ-1 larvae exhibit staining in the wing, eye and leg discs, in addition to the 35UZ pattern. In line T5 (Fig. 3C-F), staining was found in the wing disc, eye-antennal disc, and in the femur, tibia and proximal tarsus primordia of the leg discs. Little to no β-gal activity was detected in the haltere discs, in total divergence from the 35UZ pattern. T5 flies exhibit dominant leg bristle modifications on tarsal segment 2, suggesting that the transposon had disrupted a leg-patterning gene. In situ hybridization showed the T5 location of P[35UZ] to be at 35F-36A. The dachshund (dac) gene at 36A1 is involved in Drosophila eye and leg specification. dac is strongly expressed in larvae in a pattern that resembles that of T5 (Mardon et al., 1994), and genetic analysis with dac deficiencies and loss of function alleles confirmed that T5 is an allele of dac.

To check whether the transposon inserted in line T5 possessed all cis-acting sequences necessary to drive a bona fide Ubx-like pattern in larvae, we remobilized P[35UZ] at 36A. New lines were examined for lacZ patterns in imaginal discs (Table 2), and their molecular analysis included hybridization to a genomic 4.1 kb fragment located 5’ of P[35UZ] at 36A. In four lines, a second transposon was inserted in addition to P[35UZ] at 36A. All four exhibited a complex pattern consisting of both the 35UZ and dac-like patterns. Two further lines were completely deleted of lacZ sequences at 36A, but possessed a P[35UZ] (36A) derivative inserted at a new site, and both showed the 35UZ pattern. We concluded that the P[35UZ] transposon at 36A of line T5 is identical to the original transposon at 34D in 35UZ-3.

Imaginal lacZ patterns were tested for dependence on PcG regulation in mxc16a-1/Y or ph505/Y;T5/+ embryos (B) at germ band retraction reveals lacZ derepression anterior to PS6 (arrow) in ph505;T5/+ individuals; anterior is to the left and dorso-lateral up. (Fig. 3C-F).
cannot be due to alterations of the transposon, they must be attributed to new cis-regulatory sequences. We propose that in lines T5 and 35UZ-1 the P[35UZ] transposon is located close to developmental enhancer elements (of dac and fng) that drive lacZ expression in novel imaginal disc domains, and thus escape PcG regulation.

mini-white in the 35UZ lines behaves like numerous other transposons carrying both a mini-white transposition marker and a PRE: they depend on regulatory elements located both in the P[35UZ] transposon and in the flanking genomic sequences (Fauvarque and Dura, 1993; Pirrotta and Rastelli, 1994; Gindhart and Kaufman, 1995). Indeed, in adult flies, eye pigmentation patterns of six among the nine 35UZ lines are graded and uneven, and sensitive to PcG gene dosage. Several PcG gene mutations strongly increased 35UZ eye-color, whereas loss of the Ubx-regulatory elements contained in P[35UZ] and in its flanking sequences, in embryos and in larvae. But the fng-like and dac-like lacZ genes can be uncoupled at certain insertion sites.

Our data illustrate the many possible levels of interaction between regulator elements contained in P[35UZ] and in its flanking sequences. In most cases, the 35 kb of Ubx upstream sequences suffice to serve as a buffer against any influence of flanking sequences, in embryos and in larvae. But the fng-like and dac-like lacZ expression patterns in lines 35UZ-1 and T5 indicate that PcG mediated silencing depends on all cis-regulatory elements located in the vicinity of the promoter, and can be reversed when P[35UZ] is inserted near strong imaginal discs. The Contrabithorax<sup>2</sup> mutation of Ubx, that also lifts PcG-mediated gene regulation, corresponds to a similar change in flanking sequences in the endogenous locus with a breakpoint less than 10 kb upstream from the P[35UZ] fragment (Bender et al., 1985).

The sample of nine 35UZ lines does not show how often such situations occur on the chromosomes because PRE-carrying P elements are not inserted randomly in the genome (Fauvarque and Dura, 1993), and because we may have selected insertion sites where P[35UZ] expression was particularly affected during the larval stages, and hence underestimated the number of sites where PcG regulation of P[35UZ] remained intact. Indeed, such flanking sequences may silence the mini-white gene entirely. Control of endogenous Ubx expression in embryos and larvae depends on distinct enhancers (Christen and Bienz, 1994; Pirrotta et al., 1995). Ubx is activated in embryos by the segmentation genes and repressed, first by hunchback and tailless and later on by the PcG (review; Pirrotta et al., 1995). The imaginal enhancers are activated after germband retraction and drive expression in many imaginal tissues. The spatial restrictions imposed on them during the larval stages were entirely attributed to regulation by the PcG. Poux et al. (1996) suggested that a correct initial Ubx expression pattern in embryos is necessary for normal expression in imaginal tissues. Our data support this conclusion since the only line (T17) which exhibits a partially abnormal β-gal profile during embryogenesis did not show a wt pattern in larvae either. But this condition is not sufficient, as demonstrated by imaginal lacZ expression in lines 35UZ-1 and T5.

Our data further confirm that the chromatin structure imposed by PcGregulation is not irreversibly closed (Zink and Paro, 1995; McCall and Bender, 1996; Cavalli and Paro, 1998). Indeed, PcG mediated repression seems to allow a regular reassessment during Drosophila development of the activity state of all genetic elements in the vicinity of the inactivated promoter. Drosophila HOM genes include very large regulatory domains compared to many other loci, and such a reassessment mechanism could provide a possible explanation for the evolutionary origin of this particular gene structure. Indeed, one way to keep HOM gene promoters ‘out of reach’ from other enhancers activated during development could have been to surround each promoter by long stretches of DNA which helped to maintain it efficiently silenced by the PcG.

**Experimental Procedures**

**Fly strains and culture**

Flies were raised at 25°C. *multi sex comb* mutants were *mxc<sup>G43</sup>* and *mxc<sup>168</sup>* (Santamaria and Randsholt, 1995; Docquier et al., 1996).
description of other mutants and balancer chromosomes see Lindsay and Zimm (1992).

35UZ-3 and T5 remobilization

To mobilize P[35UZ] at 34D from line 35UZ-3 \(w^{118}y^{118}, 35UZ-3/35UZ-3\) females were mated to \(w^{118}y^{118}, CyO/Sp; Dr P[\Delta 2-3]/TM6\) males providing the P(\(\Delta 2-3; 99D\)) source of transposase (G0). Thirty-eight individual \(w^{118}y^{118}, 35UZ-3/CyO; Dr P[\Delta 2-3]/+, G1\) progeny were crossed to a \(w^{118}y^{118}, CDX; y^{118}y^{118}\) tester strain. In G2, a single fly with modified eye-color was selected per cross which gave rise to the T lines. For remobilization of P[35UZ] at 36A from line T5, \(w^{118}y^{118}, T5/CyO\) females were crossed to \(w^{118}y^{118}, CyO/Sp; Dr P[\Delta 2-3]/TM3\) males. In G1, \(w^{118}y^{118}, CyO/T5; Dr P[\Delta 2-3]/+\) or \(w^{118}y^{118}, Sp/T5; Dr P[\Delta 2-3]/+\) males were individually crossed to \(w^{118}y^{118}, Xa/CyO; TM3\) females. Putative new P[35UZ] lines were screened among \(w^{118}y^{118}, CyO/T5^′; TM3^+\) or \(w^{118}y^{118}, Xa/T5^′\). G2 males by a modification of the T5/+ eye-color or of the dominant T5 leg bristle phenotype. G2 males were then mated individually to \(w^{118}y^{118}, Xa;/CyO; TM3\) females.

X-gal staining

Embryos were treated as described (Docquier et al., 1996). Imaginal discs from third instar larvae were fixed in 1xPBS, 3.7% formaldehyde, washed in 1xPBS, and stained like embryos. Tissues were mounted in glycerol. ph embryo background was 25% of progeny from \(ph^{505}/FM7\) females crossed to P[35UZ] line males. Effects of PcG gene mutations on lacZ expression patterns were evaluated in imaginal discs of yellow larva from \(y^{1}mxc^{16a-1}FM7\) or \(y^{1}ph^{bx}\) females crossed to P[35UZ] males. \(ph^{505}\) clones were induced by irradiating first instar \(ph^{505+}\);35UZ-1/+ larva with 1000 rad, and examined in X-gal stained third larval instar discs.

Hybridizations

Hybridization on polytene chromosomes was according to Ashburner (1989), using a biotinylated mini-white probe. Southern hybridizations were carried out by standard procedures (Sambrook et al., 1989). Blots were revealed by autoradiography or by Phosphorimagertm (Molecular Dynamics).

P[35UZ] sequence and location analysis

Probes for characterization of P[35UZ] sequences and insertions were: 5’ and 3’ specific P-element sequences; a 1.9 kb XbaI-Clal mini-white fragment and a lacZ probe; the 41 kb Ubx promoter fragment and plasmids BXD 3105bs and PBX 3101bs (Saari and Bienz, 1987; Müller and Bienz; 1991); plasmids 2212B6 and 2212H6.5 covering a PRE and adjacent imaginal enhancers (Pirrotta et al., 1995); pEMBL19St bxds6.1,3 and PBX 3101bs (Saari and Bienz, 1987; Müller and Bienz; 1987). Long range repression conferring boundaries of the 4.1 kb Ubx  promoter fragment and plasmids

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